JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXI WASHINGTON, D. C., MAY 16, 1921 NO. 4

GLUCOSE AS A SOURCE OF CARBON FOR CERTAIN SWEET POTATO STORAGE-ROT FUNGI

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INTRODUCTION

The fact that fungi do not have the power to manufacture their own carbohydrates has long been known, and it has been demonstrated many times that carbon in certain chemical combinations is more available than in others. Some microorganisms can utilize cane sugar, while others can use only the invert sugars, and still others thrive upon organic acids, weak alcohols, and other organic compounds. Both cane sugar and invert sugars are commonly present in the sweet potato, and hence it is these two forms of carbon which should receive attention in the study of this phase of this problem.

Butkewitsch (*3*)[1] showed that *Rhizopus nigricans* Ehrb. could not utilize cane sugar as such, and, further, that this organism produced no invertase. On the contrary, Pringsheim and Zemplen (*26*) found that although *R. tonkinensis* Vuill., *Mucor javanicus* Wehm., *Penicillium purpurogenum* Fleroff., *P. africanum* Doeb., and *P. brevicaule* Sacc., could not use cane sugar directly, they did possess the power to invert it. Ritter (*28*) worked with several of the same forms and demonstrated that some of the organisms were able to invert cane sugar while others were not. He showed further that when ammonium nitrate or ammonium sulphate were employed as a source of nitrogen, nitric acid or sulphuric acid was formed, which probably inverted some of the cane sugar, thus making it available as a source of carbon for the fungi.

The investigations of Hasselbring and Hawkins (*15*) showed that the cane sugar, and to some extent the glucose content of the sweet potato in storage, increased with a decrease in starch from the time of digging in October until March or April, when there was a reverse of the process. They pointed out, also, that the amount of glucose in the sweet potato is always small as compared with that of the cane sugar. It is believed by many that the susceptibility of the sweet potato to decay likewise increases as the season progresses. This might seem to imply that there is some correlation between the sugar content of the potato and

[1] Reference is made by number (italic) to "Literature cited," p. 208–210.

its susceptibility to disease. At the time these investigations were initiated by the writers there were little available data regarding the sugar requirements of the particular fungi responsible for the decay of sweet potatoes in storage. Since glucose is probably the most readily available carbohydrate in the sweet potato for a majority of the fungi to be studied, experiments were designed to determine to what extent this sugar is utilized as a source of carbon by the following organisms: *Fusarium acuminatum* E. and E. emend. Wollenw., *Diplodia tubericola* (E. and E.) Taub., *Rhizopus tritici* Saito, *Mucor racemosus* Fes., *Sclerotium bataticola* Taub., *Penicillium* sp., *Botrytis cinerea* Pers., and *Spharonema fimbriatum* (E. and H.) Sacc.

EXPERIMENTAL METHODS

CULTURE MEDIA

Many different synthetic culture media have been used by investigators while studying the physiology of fungi. When the present investigations were begun, Czapek's nutrient solution, together with several others, were tested, but none proved entirely satisfactory. In trial experiments with *Rhizopus tritici* it was found that when ammonium nitrate was substituted for sodium nitrate and glucose for cane sugar in Czapek's solution a vigorous growth resulted. Other storage-rot fungi responded similarly. Hence, 1.6 gm. of ammonium nitrate were used instead of the 2 gm. of sodium nitrate required per 1,000 cc. in Czapek's solution. The solution as finally employed was prepared as follows:

Magnesium sulphate ($MgSO_4$)	0.5 gm.
Potassium phosphate (K_2HPO_4)	1.0 gm.
Potassium chlorid (KCl)	.5 gm.
Ferric sulphate ($FeSO_4$)	.01 gm.
Ammonium nitrate (NH_4NO_3)	1.6 gm.
Distilled water	1,000 cc.

Only C. P chemicals were used in preparing this solution.

That fungi differ in their requirements as to the source of nitrogen, as well as carbon, has been suggested by the results of many investigators. Laurent (*20*) found that *Alternaria tenuis* Nees, *Mucor racemosus* Fres, and *Aspergillus glaucus* Link, grew well on solutions containing nitrates. *Aspergillus niger* v. Tieg., on the other hand, although it can utilize nitrates as a source of nitrogen, thrives better on ammonia. It is claimed by some investigators that a combination of peptone and glucose gives the best results with most fungi. Went (*31*) found that this combination was preferable to all others for Monilia. Czapek (*5*), on the other hand, showed that the amino-acids in association with glucose are preferable to peptone for Aspergillus, and Fischer (*8*) found that *Bacillus coli* Esch, *B. subtilis* (Ehren) Cohn, and *B. pyocyanus* Gess, could use nitrate in association with glucose, but if glycerin was substituted for the glucose the latter organism alone thrived. Dox (*6*) found that species of Asper-

gillus and Penicillium grew well on Czapek's nutrient solution, which contains its nitrogen and carbon in the form of sodium nitrate and cane sugar, respectively. Young (*32*) on the other hand, used ammonium nitrate as a source of nitrogen and cane sugar as a source of carbon for *Aspergillus niger* to good advantage.

The results of various workers seem to indicate that no generalizations can be made as to the best source of nitrogen and carbon for fungi. They rather point to the fact that the various fungi differ in their requirements and that the best source of both these elements must be ascertained for each organism.

It is not claimed that the culture medium used for these investigations is the best for all the fungi studied. It is likely that a better medium might be found for each of the organisms. The nature of the work, however, required that the same substrate be used for all the fungi, and the modification of Czapek's nutrient solution as here employed seemed to meet the requirements better than any other solution tried. Only one organism (*Sphaeronema fimbriatum*) failed to thrive well upon it.

PREPARATION OF CULTURES

A sufficient quantity of medium was prepared at one time for an entire experiment, thus insuring an equitable distribution of the salts. The solution was steamed for 20 minutes in an Arnold sterilizer and then filtered to remove a slight precipitate which formed during the heating. A definite quantity by weight of this solution was then placed into each of seven large beakers, and the required amount of Baker's C. P. dextrose was added to all but one to make approximately the following strengths of the carbohydrate: 00, 10, 20, 30, 40, 50, and 60 per cent. The solutions were then steamed in the beakers for 20 minutes and again filtered. One hundred fifty cc. of each solution were added to four 300-cc. Erlenmeyer flasks previously stoppered with cotton and weighed, thus making 7 sets of 4 flasks each containing an approximately equal amount of solution with six different strengths of dextrose and one without sugar. These flasks were reweighed and sterilized by steaming for 20 minutes on three consecutive days.

INOCULATION OF CULTURES

Three flasks of each set were inoculated with a loop of a heavy spore suspension in sterile distilled water or with mycelium from young and vigorous cultures growing either on Irish potato cylinders or stems of *Melilotus alba* Desr. The fourth flask in each set not inoculated was held as a control. The cultures were incubated in the dark for two weeks at about 28° C. Notes were taken on the general character of the growth at the end of the first week and again when the experiment was terminated.

METHOD OF TAKING RESULTS

At the end of two weeks the flasks with their contents were weighed again, and the mycelium was filtered out with the aid of suction into previously burned and tared alundum crucibles. The fungous felts were washed by pouring through the crucibles a quantity of distilled water and were then dried to constant weight in a vacuum oven at 60° C. The glucose content of all the solutions was determined by means of the Fric saccharimeter.

In determining the sugar content of the 10 and 20 per cent solutions, three and two times the normal weight of the solutions, respectively, were used, while the normal weight was taken in all the higher concentrations. The solutions were then made up to 100 cc. with distilled water and polarized through a 200-mm. tube.

The control solution produced no visible polarization.

The osmotic pressure and saccharimeter readings were made with freshly filtered solutions on the day the experiment was terminated. The depression of the freezing point was determined with a Beckman thermometer in an ordinary DeWar flask, the solution being cooled by the evaporation of ether. The osmotic pressure values were calculated from the freezing points obtained by one of the two formulas given by Harris and Gortner (*14*). When these were sufficiently low, as in series I, II, III, and IV, the first formula was used and the osmotic pressure was taken from their table, or from that given by Harris (*13*). This takes into consideration the error due to undercooling, whereas the second formula does not.

The changes in acidity of the solutions were determined by the use of a potentiometer, with the arrangement of Michaelis (*23*). The measurements were made in a closed vessel provided with a dip hydrogen electrode similar to that of Bovie (*1*), except that an exit tube was provided for the hydrogen. In most cases a day or two intervened before the potentiometer readings were made. In such cases the solutions were sterilized by steaming for 20 minutes and were then kept at a temperature of about 9° C. until used. A number of preliminary tests with these and other solutions showed that the hydrogen-ion concentration was not appreciably altered by steaming.

EXPERIMENTAL DATA

PERCENTAGE OF GLUCOSE REMAINING AT THE END OF THE EXPERIMENT

As already pointed out, the culture solutions were prepared to contain glucose in concentrations from 0 to 60 per cent. In the tables which follow, the controls are the flasks of the different series which were not inoculated.

An inspection of the control columns in Table I shows how nearly the solutions made roughly to contain the same percentages of sugar agree

in the corresponding series of different experiments. The 10 per cent solutions by weight showed a range of from 9.1 per cent to 9.7 per cent at the termination of the experiments, while those made up to contain 20 per cent tested from 18.2 per cent to 19.5 per cent, and so on, as indicated in the control columns in each series and under each organism.

TABLE I.—*Percentage of glucose remaining in the solutions at the end of the experiment* [a]

[Saccharimeter readings]

Series No.	Percentage of glucose by weight.	Solutions.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*	*Sclerotium batatícola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control....	0	0	0	0	0	0	0	0
		Inoculated.	0	0	0	0	0	0	0	0
II	10	Control....	9.7	9.4	9.5	9.4	9.6	9.26	9.3	9.1
		Inoculated.	8.55	1.2	8.3	5.2	2.2	3.16	8.83	9.1
III	20	Control....	18.85	18.5	19.35	19.5	18.95	18.6	18.75	18.2
		Inoculated.	17.9	14.7	17.71	15.8	14.16	15.53	17.7	18.2
IV	30	Control....	28	26.8	29.2	28.7	27.8	28.1	28.5	25.5
		Inoculated.	27.1	23.3	27.2	26	25.4	23.46	26.03	25.5
V	40	Control....	37.1	38	39.7	38.1	37.3	37.5	37.8	37
		Inoculated.	36.7	37.3	36.8	36.9	36.7	32.86	37.8	.....
VI	50	Control....	46.1	48	49.2	47.8	45.5	46.9	47.7	.....
		Inoculated.	46.8	48.3	46.7	48.1	45.7	44.86	.....	.....
VII	60	Control....	57.5	57.3	56.4	58.8	57.1	57.9	.....	.....
		Inoculated.	.....	.....	.....	.....	.....	57	.....	.....

[a] Column 2 shows the percentage of glucose by weight, while the actual percentage of sugar present at the end of the experiments in each case is indicated by the saccharimeter readings given in the control columns. The significance of column 2 is the same in all the following tables.

For the sake of convenience in some of the general discussions reference will be made to the approximate percentages as shown in column 2 instead of to the actual percentage based on the saccharimeter readings.

The column headed "inoculated" represents the average of the concentrations of the three inoculated flasks of each series at the end of the experiment. For example, in series II of the *Fusarium acuminatum* experiment the control tested 9.7 per cent, while the average of the three inoculated flasks is 8.55 per cent. The control in the Diplodia experiment of series II tested 9.4 per cent, while the inoculated flasks averaged 1.2 per cent of glucose. Similarly the amount of glucose in the controls and in the flasks in which the organisms have grown may be contrasted with all of those in the same series in all the experiments.

The results show that all these fungi have the power to germinate and grow in a solution of glucose of high osmotic concentration. As early as 1889 Eschenhagen (7) showed that *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea* Pers. could grow in solutions of glucose of 53, 55, and 51 per cent strength, respectively, and Raciborski (*27*) found that *A. glaucum* and a species of Torula could grow in a salt solution of even greater concentration. Hawkins (*16*) likewise showed that various species

of fungi could thrive in concentrated solutions of glucose ranging from 1.6 to 2.6 molecular and 1.6 and 1.8 molecular sucrose.

With two exceptions the fungi studied by the writers grew on solutions of glucose from approximately 42 to 50 per cent concentration. The two exceptions, *Botrytis cinerea* and *Sphaeronema fimbriatum*, made a fair growth on solutions of approximately 38 and 25 per cent, respectively.

The actual loss in the amount of sugar is somewhat significant and differs considerably with the different fungi. The greatest consumption, if it may be so expressed, occurs in the 10 per cent concentration. In a 10 per cent concentration *Diplodia tubericola* reduced the percentage of sugar from 9.4 to 1.2; *Mucor racemosus* from 9.4 to 5.2; *Sclerotium bataticola* from 9.6 to 2.2; and *Penicillium* sp. from 9.26 to 3.16. In general, the percentage of sugar actually consumed by the organisms just named decreased as the concentration of the solutions increased. On the other hand, *Rhizopus tritici* and *Botrytis cinerea* used more sugar at the higher concentrations, while *Sphaeronema fimbriatum* produced no change.

TOTAL AMOUNT OF GLUCOSE PRESENT

Although the study of Table I shows quite clearly that considerable glucose is converted by the action of some of the fungi, yet this does not show exactly what has occurred, since the figures are expressed in percentages and certain factors are not considered. For example, no account was taken of the amount of evaporation or of the difference in the quantity of solution present in the flasks. Table II shows more accurately the amount of sugar remaining in each flask at the end of the experiment. These figures were obtained by multiplying the number of grams of dextrose in each 100 gm. of solutions—namely, percentage of dextrose present—by the total number of grams of the medium present and dividing the result by 100. The amount of substrate in each flask was determined by deducting the combined weight of the flask and dried fungous growth from that of the solution, flask, and mycelium. In the *Rhizopus tritici* experiment, which was the first one conducted, not enough solution of all strengths was prepared to permit of the addition of 150 cc. to each flask; hence the control flask in some cases contained 100 cc. or even less. This causes what would at first appear to be a great inconsistency in the amount of dextrose present in the control as compared with that in the inoculated flasks. This is especially evident in series VI, where the total amount of dextrose in the control was 41.82, while the average for the inoculated flasks was 78.74. It is, however, also noticeable in series III, IV, and V.

TABLE II.—*Amount of glucose remaining in the solutions at the end of the experiments*

[Expressed in grams]

Series. No.	Percentage of dextrose by weight.	Solution.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus trilici.*	*Mucor racemosus.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control	14. 54	14. 26	12. 96	14. 34
		Inoculated	12. 80	1. 79	12. 33	7. 60
III	20	Control	29. 48	28. 84	23. 39	30. 40
		Inoculated	27. 69	22. 25	26. 94	24. 00
IV	30	Control	45. 50	44. 14	33. 11	46. 69
		Inoculated	43. 78	36. 75	42. 89	40. 71
V	40	Control	63. 48	63. 80	36. 40	64. 85
		Inoculated	62. 34	62. 57	60. 09	61. 43
VI	50	Control	81. 32	85. 34	41. 82	84. 27
		Inoculated	82. 38	85. 25	78. 74	84. 29
VII	60	Control	107. 35	104. 92	100. 90	106. 84
		Inoculated	.....	.....	.....	.....

Series No.	Percentage of dextrose by weight.	Solution.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control	14. 48	13. 72	13. 73	13. 61
		Inoculated	3. 19	4. 53	13. 04	13. 53
III	20	Control	29. 68	28. 89	29. 10	28. 21
		Inoculated	21. 16	23. 37	27. 28	28. 48
IV	30	Control	45. 45	45. 44	46. 14	41. 34
		Inoculated	40. 13	36. 22	41. 50	41. 28
V	40	Control	62. 93	63. 30	63. 62	.....
		Inoculated	60. 74	53. 29	62. 99	.....
VI	50	Control	79. 62	84. 04	84. 05	.....
		Inoculated	79. 25	77. 64	.....	.....
VII	60	Control	.....	105. 96	.....	.....
		Inoculated	.....	104. 62	.....	.....

From Table II it is seen that in no case was all the sugar used up, although the total amount remaining in some cases was small, *Diplodia tubericola*, *Sclerotium bataticola*, and *Penicillium* sp. being the heaviest users.

TOTAL AMOUNT OF GLUCOSE PRESENT AT THE BEGINNING OF THE EXPERIMENTS

In order to determine the amount of glucose utilized by the different fungi it was necessary to calculate the total amount present in each flask at the beginning of the experiments. Data for making these calculations were available, since the total amounts of glucose in the controls were the same at the beginning as at the end of the experiments and the concentrations of the sugar in the inoculated flasks and controls were originally identical.

The figures in Table III show the total amount of glucose present in the control at the beginning of the experiment and the average amount

in the three inoculated flasks of each series. It will be seen that in most cases the amounts in the inoculated flasks averaged very nearly the same as that present in the controls. It must be remembered also that although the solution originally was of a uniform concentration and that 150 cc. were placed in each flask, yet the weight of the contents of the flasks varied slightly, because of the difficulty of measuring accurately with a graduate, which accounts for the difference in the total sugar present.

TABLE III.—*Total calculated amount of glucose present in the solutions at the beginning of the experiments*

[Expressed in grams]

Series No.	Percentage of dextrose by weight.	Solutions.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control	14.54	14.26	12.96	14.36
		Inoculated	14.58	14.25	14.05	14.23
III	20	Control	28.48	28.84	23.39	30.40
		Inoculated	29.42	29.13	29.66	30.39
IV	30	Control	45.50	44.14	33.11	46.69
		Inoculated	45.59	43.79	46.23	45.92
V	40	Control	63.48	63.80	36.40	64.85
		Inoculated	63.15	64.55	64.79	62.42
VI	50	Control	81.32	85.34	41.82	84.27
		Inoculated	81.24	84.78	82.05	84.21
VII	60	Control	107.35	104.92	100.90	106.84
		Inoculated				

Series No.	Percentage of dextrose by weight.	Solutions.	*Sclerotium batalicola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control	14.48	13.72	13.73	13.61
		Inoculated	14.47	13.72	13.81	13.53
III	20	Control	29.68	28.89	29.10	28.21
		Inoculated	29.68	28.84	29.14	28.44
IV	30	Control	45.45	45.44	46.14	41.34
		Inoculated	45.32	43.12	46.15	41.13
V	40	Control	62.93	63.30	63.62	
		Inoculated	62.50	63.23	63.23	
VI	50	Control	79.62	84.04	84.05	
		Inoculated	79.65	82.77		
VII	60	Control		105.96		
		Inoculated		106.38		

TOTAL AMOUNT OF GLUCOSE REDUCED

The amount of glucose present in the solutions both at the beginning and at the end of the experiments having been determined, the differences between these two or the amount reduced by the fungi were calculated and are shown in Table IV. An examination of this table

shows some very interesting results. In discussing these results it must be borne in mind that all the glucose which has disappeared from the solution has not been actually utilized in the formation of the fungous material. Certain compounds, such as alcohol, organic acids, and carbon dioxid probably were formed.

TABLE IV.—*Total amount of glucose reduced*

[Expressed in grams]

Series No.	Percentage of dextrose by weight.	Solution.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control				
		Inoculated	1.78	12.45	1.73	6.30
III	20	Control				
		Inoculated	1.73	6.89	2.72	6.38
IV	30	Control				
		Inoculated	1.81	7.04	3.34	5.18
V	40	Control				
		Inoculated	.81	1.67	4.70	2.99
VI	50	Control				
		Inoculated	.06	0	3.31	.09
VII	60	Control				
		Inoculated	0	0	0	0

Series No.	Percentage of dextrose by weight.	Solution.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control				
		Inoculated	11.30	9.18	0.77	
III	20	Control				
		Inoculated	8.51	5.47	1.85	0
IV	30	Control				
		Inoculated	5.18	6.90	4.65	
V	40	Control				
		Inoculated	1.76	9.60	.25	0
VI	50	Control				
		Inoculated	.40	5.15	0	0
VII	60	Control				
		Inoculated	0	1.76		

In concentrations of approximately 10 per cent, four organisms, *Diplodia tubericola*, *Sclerotium bataticola*, *Penicillium* sp., and *Mucor racemosus*, have reduced a considerable quantity of the total amount of glucose originally present. All these fungi utilize a larger amount of sugar at a strength of 10 per cent than at any higher concentration, except *Penicillium* sp., which reduced slightly more in the 40 per cent solution. Penicillium alone grew and consumed some glucose in an approximately 58 per cent solution. With these organisms *Rhizopus tritici* and *Botrytis cinerea* should especially be compared. The two latter fungi used a comparatively small amount of sugar at any concentration tried. It is, furthermore, significant that they used the lesser amount at the lower concentration, the quantity consumed increasing with the increase of

the concentration up to 40 and 30 per cent, respectively, followed by a reverse of the process.

With the exception of *Penicillium* sp. none of the organisms grew in an approximately 60 per cent solution. Two fungi, *Diplodia tubericola* and *Botrytis cinerea*, would not grow in a 50 per cent solution, and *Sphaeronema fimbriatum* practically not at all at any concentration. It would appear from this table alone that a 10 per cent solution was the optimum concentration for growth of *D. tubericola* and *Sclerotium bataticola;* for *Rhizopus tritici* and *Penicillium* sp., 40 per cent; *B. cinerea*, 30 per cent. *Fusarium acuminatum* and *Mucor racemosus* do about equally well in concentrations of 10, 20, and 30 per cent. Whether or not Table IV gives a fair index of the optimum concentration for growth will be better understood after studying Tables V and VI.

DRY WEIGHT OF MYCELIUM PRODUCED

The dry weight produced by the various organisms in each flask was obtained separately, and the average weight from three flasks of each series is shown in Table V.

TABLE V. *Dry weight of mycelium*

[Expressed in grams]

Series No.	Percentage of dextrose by weight.	Solutions.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control				
		Inoculated	0.0150	0.0350	0.0300	0.0131
II	10	Control				
		Inoculated	.1094	1.4750	.4716	.3375
III	20	Control				
		Inoculated	.1352	1.0692	.6516	.2519
IV	30	Control				
		Inoculated	.2049	1.6045	.6464	.1906
V	40	Control				
		Inoculated	.1329	.8898	.3660	.1996
VI	50	Control				
		Inoculated	0	.1835	.1915	.2433
VII	60	Control				
		Inoculated	0	0	0	0

Series No.	Percentage of dextrose by weight.	Solution.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control				
		Inoculated	0.0451	0.1682	0.0778	0.0126
II	10	Control				
		Inoculated	2.2482	2.6106	.2743	.0191
III	20	Control				
		Inoculated	1.8194	2.0308	.9154	.0239
IV	30	Control				
		Inoculated	1.4936	2.4063	1.0215	.0103
V	40	Control				
		Inoculated	.4858	2.1210	.0811	0
VI	50	Control				
		Inoculated	.1114	1.7470	0	0
VII	60	Control				
		Inoculated	0	.2717	0	0

In considering the dry weight of mycelium produced, one must bear in mind that the growth of fungi in cultures of the same composition varies greatly. The dry weights given in Table V are the averages of of the growth from three flasks. It frequently happened during the course of these experiments that one culture produced a small amount of mycelium when compared with the other two flasks of the same series. Had it been possible to obtain the average weight from a large number of cultures, the paucity of growth of a single one would not have influenced the average so much.

Kunstmann (*18*) in working with *Aspergillus niger* and *Penicillium glaucum* on sugar solutions found that in extreme cases there were five or more times as much dry material in one culture as in another of the same composition and incubated under identical conditions.

A small amount of mycelium was produced in cultures with no glucose added. *Diplodia tubericola*, *Sclerotium bataticola*, and *Penicillium* sp. produced considerably more dried material than any of the other organisms, especially at concentrations of approximately 10, 20, and 30 per cent; and, as was previously suggested, these were the organisms which utilized in general the largest amount of glucose. *Sphaeronema fimbriatum*, which did not use a determinable amount of sugar, produced a smaller amount of mycelium than any other organism. The dry weight produced in all of the series is, within the limits of experimental error, about equal to the amount produced in the control.

GLUCOSE REQUIRED TO PRODUCE 1 GM. OF DRY WEIGHT

The amount of glucose reduced for each gram of dry weight formed is shown in Table VI. It must be remembered that these figures do not represent necessarily the quantity of glucose which was actually utilized by the fungus in respiration or in producing dry weight, but the amount changed so that it no longer possessed polarizing properties. Doubtless some of the sugar was consumed in forming various compounds resulting from incomplete respiration or other vital activities.

TABLE VI.—*Grams of glucose reduced for each gram of dry weight formed*

Series No.	Percentage of dextrose by weight.	Solution.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus trilici.*	*Mucor racemosus.*
I	0	Control				
		Inoculated	0	0	0	0
II	10	Control				
		Inoculated	17.52	8.78	3.70	19.65
III	20	Control				
		Inoculated	12.60	6.59	4.17	25.56
IV	30	Control				
		Inoculated	9.07	4.99	5.17	28.88
V	40	Control				
		Inoculated	6.38	2.13	12.91	15.89
VI	50	Control				
		Inoculated			17.67	1.44
VII	60	Control				
		Inoculated				

TABLE VI.—*Grams of glucose reduced for each gram of dry weight formed*—Continued

Series No.	Percentage of dextrose by weight.	Solution.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control				
		Inoculated	1 0	0	0	0
II	10	Control				
		Inoculated	5. 02	3. 58	2. 68	
III	20	Control				
		Inoculated	4. 59	2. 70	2. 07	
IV	30	Control				
		Inoculated	3. 39	2. 93	4. 56	
V	40	Control				
		Inoculated	3. 86	5. 23	5. 82	
VI	50	Control				
		Inoculated	2. 98	3. 05		
VII	60	Control				
		Inoculated		8. 61		

In the same concentration of sugar the different organisms varied greatly in the amount of glucose required to produce 1 gm. of dry weight. Likewise the amount required to produce 1 gm. of dry weight of the same fungus differs greatly with the concentration of the solution. For example, *Fusarium acuminatum* required a little over 6 gm. of glucose to make 1 gm. of dry weight in an approximately 40 per cent solution. On the other hand, in a 10 per cent solution over 17 gm. were required, or nearly three times as much. In other words, the higher the concentration the smaller the amount of glucose used to form 1 gm. of dry weight. Within the limits of these experiments the "economic coefficient" of *F. acuminatum* in a 40 per cent solution would be 6.38. *Diplodia tubericola* forms a curve similar to that of *F. acuminatum*, but the quantity of glucose required to produce 1 gm. of dry weight is considerably less in corresponding concentrations of the solution, its "economic coefficient" in about the same concentration being 2.13. *Rhizopus tritici* reverses the process, the lowest "economic coefficient" of 3.70 being in an approximately 10 per cent concentration. In a 50 per cent solution the same fungus utilized 17.67 gm. of glucose to produce 1 gm. of dry material. If *R. tritici* is compared with *Mucor racemosus*, which required 1.44 gm. of sugar to produce 1 gm. of dry weight, it is seen that the order is again reversed, the lowest "economic coefficient" occurring in a concentration of 50 per cent. The highest "economic coefficient" of the latter organism was in a 28.7 per cent solution and increased both above and below this concentration. *Sclerotium bataticola* parallels *M. racemosus* and in every case except in a 50 per cent solution requires much less sugar to form 1 gm. of dried material. Neither *Penicillium* sp. nor *Botrytis cinerea* is quite consistent, in that the minimum glucose required per gram of dry weight is not at either end of the series.

A comparison of the maximum amount of glucose required to form 1 gm. of dry weight shows remarkable variations among the different

fungi. *Mucor racemosus*, requiring 28.88 gm., stands at one extreme and *Sclerotium bataticola*, requiring 5.02 gm., at the other. *Fusarium acuminatum*, *Diplodia tubericola*, and *Rhizopus tritici* required 17.52, 8.78, and 17.67 gm., respectively. Pfeffer (*25*) and Kunstman (*18*) have termed the numerical relation between the sugar consumed and the dry weight of the substance formed the "economic coefficient." The minimum amount of sugar required to produce 1 gm. of dry weight shows likewise great variation. A comparison of *F. acuminatum* and *M. racemosus*, the two extremes, shows that the "economic coefficient" of the former is about 4.4 times greater than that of the latter. These differences correspond fairly well with results obtained by Kunstmann working with *Aspergillus niger* and *Penicillium glaucum*, who found it to vary from 1.13 to 3.88, while Ono (*24*) obtained a value as high as 6.1 when working with *A. niger*.

These results indicate that the coefficient cannot be looked upon as in any way constant. It differs with different fungi grown under identical conditions. There may be several factors which influence the results. According to Jost (*17*), the coefficient increased with the progressive development of the fungus and with the elevation of the temperature. Toxic substances likewise were shown by Ono to increase the coefficient in Aspergillus. Since these organisms were all grown at the same temperature, it would be largely eliminated as a controlling factor. On the other hand, some of the chemicals or impurities composing the nutrient medium might have a toxic action on some of the organisms. Furthermore, it is likely that the different fungi vary greatly in their ability to form other substances, such as alcohol, organic acids, etc., which might be toxic or inhibit normal growth. In some cases it has been shown that alcohol is actually produced. That all organisms are not equally influenced by these changes is probably true. In the more resistant forms growth would continue for a longer time and thus possibly produce a greater amount of fungal material per unit volume of sugar. What changes the fungus actually brings about must remain unknown until chemical methods permit of the quantitative determinations of the different products and materials formed.

HYDROGEN-ION CONCENTRATIONS

The hydrogen-ion concentrations express in P_H values were determined from representative samples of all the solutions. These are recorded in Table VII.

TABLE VII.—*Hydrogen-ion concentrations, expressed in terms of potential hydrogen*

Series No.	Percentage of dextrose by weight.	Solution.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control	4. 12	4. 41	6. 33	6. 54
		Inoculated	4. 24	3. 89	6. 12	6. 02
II	10	Control	3. 97	3. 97	4. 84	5. 44
		Inoculated	3. 89	3. 66	2. 11	3. 09
III	20	Control	3. 93	3. 89	4. 50	5. 06
		Inoculated	3. 71	2. 52	1. 90	3. 21
IV	30	Control	3. 96	3. 91	4. 20	4. 30
		Inoculated	3. 66	2. 30	1. 81	3. 46
V	40	Control	4. 22	3. 75	4. 10	4. 39
		Inoculated	3. 62	2. 27	2. 00	3. 13
VI	50	Control	3. 97	4. 07	4. 00	4. 28
		Inoculated	3. 99	3. 84	2. 81	4. 17
VII	60	Control	4. 09	3. 72	4. 12	4. 08
		Inoculated	.....	.....	.....	.....

Series No.	Percentage of dextrose by weight.	Solution.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	6. 49	6. 44	6. 39	6. 41
		Inoculated	5. 15	6. 41	6. 25	6. 41
II	10	Control	4. 54	5. 70	4. 99	5. 19
		Inoculated	4. 21	4. 37	3. 24	5. 50
III	20	Control	4. 83	5. 39	4. 51	5. 16
		Inoculated	4. 06	3. 81	2. 91	5. 05
IV	30	Control	4. 80	5. 14	4. 22	4. 72
		Inoculated	4. 07	3. 92	3. 45	4. 83
V	40	Control	4. 82	4. 79	4. 07	4. 52
		Inoculated	3. 69	3. 44	3. 92	.....
VI	50	Control	4. 76	4. 51	4. 00	.....
		Inoculated	4. 75	3. 29	.....	.....
VII	60	Control	4. 75	4. 40	.....	.....
		Inoculated	.....	3. 16	.....	.....

No attempt was made to bring the different solutions to a definite or uniform hydrogen-ion concentration. What influence the organisms had on the hydrogen-ion concentration was determined by a comparison of the controls with the solutions after the removal of the fungous growth. That the solutions differed somewhat in their original hydrogen-ion concentration is seen from Table VII. Gillespie (*11*) in working with different strains of the potato scab organism, *Actinomyces chromogenus* Gasp. in culture media of different composition found that the growth was slower and less vigorous in a solution with a hydrogen-ion concentration of P_H 5.2 than in less acid media. It would seem that, although there was some variation as regards the tolerance of the different strains to acid media, a P_H of 5.2 would closely approximate the limit of growth. A further interesting fact in this connection also was brought out by Gillespie, who showed that cultivated soils of the Caribou loam, which is generally free from the scab organism, yield a water extract with P_H values varying from 4.9 to 5.5 with a mean of 5.2. Meacham (*22*) found

that the growth of *Lenzites sepiaria* (Wulf.) Fr., *Fomes roseus* (Alb. and Schw.) Fr., *Coniophora cerebella* (Pers.) Schröter, and *Merulius lachrymans* (Jcq.) Fr. in synthetic and malt extract media was not inhibited until a high hydrogen-ion concentration was reached. The limiting P_H value was found to be near 1.7. All the fungi responded in about the same way, although there were distinct variations between the different organisms. Growth appeared to be retarded when a P_H value of about 3.0 was reached. As the acidity increased beyond this point the growth became markedly less.

Webb (*29*) found that in a culture solution consisting of mannite, phosphoric acid, and sodium hydroxid successively increasing the hydrogen-ion concentrations from approximately neutral to P_H 3.1 to 2.8 influenced favorably the germination of the spores of *Aspergillus niger*, *Penicillium cyclopium* Westl., *Botrytis cinerea*, *Fusarium* sp., and *Lenzites sepiaria*. The different organisms had a different range of hydrogen-ion concentration, permitting the germination of the spores as follows: *A. niger*, P_H 2.8 to 8.8; *P. cyclopium* 2.8 to 10+; *B. cinerea* 2.8 to 7; *Fusarium* sp., 2.8 to 10+; and *L. sepiaria* 2.8 to 7.

At certain concentrations some of the organisms studied by the writers had little or no influence on the acidity of the substrate, while others rendered it much more acid. For example, *Fusarium acuminatum*, *Sclerotium bataticola*, and *Sphaeronema fimbriatum* can hardly be interpreted as exercising any influence on the acidity of the solution within the limits of these experiments. With all three of these fungi the hydrogen-ion concentration in the control at the same concentrations is actually higher than the solution on which the fungus grew. The differences are slight and in some cases well within the limits of experimental error, so that in general there are no outstanding examples of increase or decrease in acidity of any one of these three fungi. On the other hand, the remaining five organisms all show a considerable increase in the hydrogen-ion concentration in solutions of different strength. *Rhizopus tritici*, for example, increased the acidity of the substrate from P_H 4.2 to 1.81 in a solution of 30 per cent dextrose, when the maximum hydrogen-ion concentration was reached. In general, it may be said that the highest concentration was reached in solutions containing about 20 to 30 per cent glucose and that it decreased above and below these strengths.

Just how nearly these results represent the limits of growth for these fungi is not definitely known. The greatest growth was made during the first week; thereafter it slowed up. Whether or not this was due to the increase of acidity in the solution or to other causes can not be answered at the present time.

Preliminary experiments showed that if *Rhizopus tritici* is grown several times on the same solution after removal of the fungus felt it finally reaches a stage when growth no longer will take place. These experiments were made with Czapek's modified nutrient solution with a 2 and 1 per

cent of glucose and starch, respectively, as a source of carbon and with sweet potato bouillon. The fungus was allowed to grow 10 days, when the felt was removed and the dry weight determined. The Czapek's nutrient solution had a hydrogen-ion concentration of P_H 3.07 previous to inoculation. After a 10 days' growth the acidity had increased to P_H 2.05. The solution was sterilized by autoclaving and was then reinoculated with the same organism, but no growth resulted. Repeated inoculations were made, but no growth resulted. No appreciable increase or decrease in the P_H value of the solution resulted from the autoclaving either in Czapek's solution or in the sweet potato bouillon. Although the fungus would not grow, there remained a considerable quantity of glucose and starch in the solution. In a 2-liter flask containing 1,000 cc. of solution 1.28 gm. of dried mycelium were produced.

The sweet potato bouillon had an original P_H value of 5.17, which was increased by a 10 days' growth to 3.12. The dry weight produced on 1,000 cc. of solution in a 2-liter flask amounted to 3.59 gm. The solution was sterilized by autoclaving and was reinoculated. At the end of 10 more days the P_H value was 3.08 and the dry weight was 1.33 gm. This operation was twice more repeated, and the P_H values after two more periods of 10 days' growth were 3.11 and 3.05, respectively. The dry weight of the mycelium in the former case was 0.2536 gm., and not enough was produced in the latter to determine. Glucose and starch still remained in the solution. It is seen that the hydrogen-ion concentration did not increase materially after the first 10 days' growth, while the actual amount of dry weight of material greatly decreased.

OSMOTIC CONCENTRATIONS OF THE SOLUTIONS

Table VIII shows the osmotic pressures in atmospheres of the sugar solutions at the end of the experiments. These values were determined for the first five series of each organism. There was so little growth in the highest concentrations that they were not considered of sufficient importance to justify the determination of their osmotic pressures.

Attention was previously called to the fact that fungi can germinate and grow in a sugar or salt solution far more concentrated than that of the cell sap of its host. The literature is replete with instances of certain fungi, such as species of Penicillium and Aspergillus, which are able to grow on concentrated sugar solutions. All the organisms discussed in this paper will germinate on a solution of glucose with an osmotic concentration several times greater than that of the cell sap of the sweet potato.

An examination of Table VIII shows that these fungi grew in solutions with a maximum osmotic pressure varying from 81.33 to 101.46 atmospheres. It will be noticed also that in general *Fusarium acuminatum* and *Mucor racemosus* increased, while all the other organisms decreased the osmotic concentration of the solutions during a period of two weeks' growth. Some exceptions in this connection should be noted. *F.*

acuminatum decreased the osmotic concentration in Czapek's modified nutrient solution without sugar added more than any of the other organisms. *Sclerotium bataticola* and *Botrytis cinerea* decreased the concentrations in all except 40 per cent solutions, where there was a slight increase. The decrease in the osmotic pressure is especially marked with *S. bataticola* and *Penicillium* sp. in the 10 per cent solution, being reduced in the former from 16.12 to 9.31 atmospheres and in the latter from 16.48 to 6.26 atmospheres.

TABLE VIII.—*Osmotic pressure of the sugar solutions*

[Expressed in atmospheres]

Series No.	Percentage of dextrose by weight.	Solutions.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus trilici.*	*Mucor racemosus.*
I	0	Control	2.41	2.17	1.93	2.29
		Inoculated	1.93	2.01	1.81	2.29
II	10	Control	16.37	16.24	16.36	16.84
		Inoculated	17.24	12.52	14.44	19.04
III	20	Control	.....	33.36	34.79	35.75
		Inoculated	.....	31.09	32.41	39.53
IV	30	Control	66.76	53.37	62.03	59.30
		Inoculated	68.73	53.17	58.27	63.52
V	40	Control	101.46	92.20	98.12	93.85
		Inoculated	101.89	92.59	95.64	98.51
VI	50	Control	.....	.....	.....	.....
		Inoculated	.....	.....	.....	.....
VII	60	Control	.....	.....	.....	.....
		Inoculated	.....	.....	.....	.....

Series No.	Percentage of dextrose by weight.	Solutions.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	2.17	1.93	1.93	1.93
		Inoculated	2.13	1.67	1.89	1.85
II	10	Control	16.12	16.48	15.76	15.88
		Inoculated	9.31	6.26	14.84	15.96
III	20	Control	33.96	34.68	33.36	33.60
		Inoculated	30.89	27.86	31.45	33.72
IV	30	Control	55.03	58.47	58.00	52.06
		Inoculated	54.04	48.02	54.75	52.01
V	40	Control	87.98	81.33	90.68	81.62+
		Inoculated	90.92	80.39	92.12	.....
VI	50	Control	.....	.....	.....	.....
		Inoculated	.....	.....	.....	.....
VII	60	Control	.....	.....	.....	.....
		Inoculated	.....	.....	.....	.....

Why some organisms increase and others decrease the osmotic concentration of the solution is not definitely known. It is difficult to explain why an organism decreases the osmotic pressure in all strengths of the sugar solution except in the 40 per cent and there slightly increases it. In this connection *Sclerotium bataticola* and *Botrytis cinerea* should be especially noted.

In seeking for an explanation of the increase or decrease of the osmotic concentration by the different organisms in solutions containing the same percentage of sugar, one should take into consideration the cleavage products formed. Many investigators have shown that some fungi, and it may apply to all, have the power to produce organic acids, alcohol, etc. Wehmer (*30*) gave the generic name Citromyces to a group of fungi which he believed to be characterized by their ability to produce citric acid. Oxalic acid fermentation was thought by him to be a characteristic of *Aspergillus niger*. Currie (*4*) some years later showed that citric acid was likewise produced by *A. niger* in nutrient solutions. Lind (*21*) showed that oxalic acid was produced by *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea*, and Lafar (*19, p. 351*) by *A. niger*. Lafar also found that citric acid was formed by *Citromyces pfefferianus* Wehm., *C. glaber* Wehm., and *P. luteum*. Many other references might be cited to show that a great variety of fungi and bacteria produce acids of various sorts.

The production of alcohol has been proved to be a regular phenomenon of many fungi, the amount formed depending on the substrate, the temperature at which incubated, and the length of the incubation period.

Gayon (*10*), Hansen (*12*), Fitz (*9*), and Brefeld (*2*) showed that *Mucor spinosus* v. Tieg. and *Rhizopus nigricans* produced alcohol in varying amounts, depending somewhat upon the conditions mentioned above. *M. racemosus* produced alcohol but little when incubated at a temperature below 15° C. *R. nigricans* ceased to produce alcohol when 1.5 per cent had been formed. *M. spinosus*, according to Gayon, will produce as much as 1.5 to 2 per cent, and *M. erectus* Bain. as much as 8 per cent, according to Hansen, when incubated at room temperature. If either alcohol or acids or both were formed in the solutions the osmotic concentration would be influenced in proportion to the amount produced. Previous tables have shown that the percentage of sugar was lowered by the fungus, and yet in spite of that the osmotic concentration of some of the solutions is actually higher than that of the controls. This would be possible only in case some of the sugar or other constituents of the substrate were converted into some substance, such as alcohol or organic acids, which would affect the osmotic concentration. In *M. racemosus* the osmotic pressure of the solution of the inoculated flask is higher than in the control, although a considerable amount of the sugar was actually removed. This organism is one which is known, as pointed out in one of the citations given above, to produce a considerable quantity of alcohol. Data presented in Table II show that *Diplodia tubericola*, *M. racemosus*, and *Sclerotium bataticola* growing in an approximately 10 per cent solution reduced the sugar from 14.26, 14.34, and 14.48 to 1.29, 7.60, and 3.18 gm., respectively. On the other hand, the osmotic pressures of these solutions were changed from 16.24, 16.84, and 16.12

to 12.52, 19.04, and 9.31, respectively. In the first and last cases the osmotic pressure is reduced, the latter more than the former, but in neither case in proportion to the amount of sugar actually disposed of. In *M. racemosus*, which reduced the sugar by nearly one-half, the osmotic pressure was actually increased. A study of the two tables will show other similar results. These facts may be explained in part by assuming that some of the sugar was broken down into some substance or substances which held the osmotic concentration up to, or in some cases raised it above, that of the control.

CHARACTER OF MYCELIAL GROWTH

The fungi all looked very much alike when grown upon the culture medium without glucose present. The mycelium was white or grayish in color, very fine, and usually formed a thin felt over the surface with a small amount of submerged threads. Except in *Rhizopus tritici*, no sporulation took place, and then only an occasional sporangium was formed. With two exceptions, all the fungi studied made a fairly good growth in concentrations of glucose varying roughly from 10 to 40 per cent. Sphaeronema apparently is not able to utilize glucose, since in no case was the growth perceptibly increased by its presence. Of the eight fungi, Penicillium alone was able to grow in the strongest concentration. *Diplodia tubericola*, *R. nigricans*, *Mucor racemosus*, and *Sclerotium bataticola* made some growth in all but series VII, while *Fusarium acuminatum* made a sparse growth in series VI. *Sphaeronema fimbriatum* and *Botrytis cinerea* failed to thrive in series V and VI, respectively. Although Penicillium grew at the highest concentration used, it did not develop normally. No doubt this strength of dextrose represents approximately the limit of growth of this organism.

SUMMARY

(1) Eight fungi—*Fusarium acuminatum*, *Diplodia tubericola*, *Rhizopus tritici*, *Mucor racemosus*, *Sclerotium bataticola*, *Penicillium* sp., *Botrytis cinerea*, and *Sphaeronema fimbriatum*—which cause decay of sweet potatoes in storage, were grown at a constant temperature of 28° C. on a modification of Czapek's nutrient solution, with different amounts of glucose as a source of carbon. All these fungi except *S. fimbriatum* utilized glucose in considerable amounts.

(2) The different fungi varied greatly in the amount of glucose they actually consumed at the same concentration. In general, the greatest consumption was in the weaker solution (10 per cent) and decreased progressively with the increase of the concentration. With two exceptions, all the organisms grew in solutions containing from 42 to 50 per cent glucose. *Penicillium* sp. alone grew in a 58 per cent solution.

(3) A great variation was found among the different fungi in the amount of dry material that was produced at the same concentration.

The concentration on which the greatest yield of fungous material was produced by a certain organism was not necessarily the optimum concentration for any of the other fungi.

(4) The different organisms varied greatly in the amount of glucose required to produce 1 gm. of dry weight. Likewise the amount required to produce 1 gm. of dry weight of the same fungus differed with the concentration of the solution.

(5) The "economic coefficient" was found to be much higher in many cases than that given by Kunstmann and Ono; the maximum of 28.88 and the minimum of 1.44 being reached by *Mucor racemosus* on a 30 and 50 per cent solution, respectively. The highest "economic coefficient" for some fungi was on the weaker solutions. For other organisms, however, the order was reversed.

(6) Some of the organisms—namely, *Fusarium acuminatum*, *Sclerotium bataticola*, and *Sphaeronema fimbriatum*—had little or no influence on the hydrogen-ion concentration. *Rhizopus tritici*, *Diplodia tubericola*, *Mucor racemosus*, *Penicillium* sp., and *Botrytis cinerea*, on the other hand, increased perceptibly the acidity of the solution.

(7) All of the fungi studied grew in solutions with a maximum osmotic pressure varying from 81.33 to 101.46 atmospheres. *Fusarium acuminatum* and *Mucor racemosus* increased the concentration, whereas, the other fungi in general decreased it. In a few cases where a considerable amount of sugar was consumed the concentration was actually increased. In general, the decrease in the osmotic concentration was not in proportion to the sugar consumed, so that it is possible that compounds such as organic acids, alcohol, etc., were formed from the sugar which would themselves influence the osmotic concentration.

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RESPIRATION OF SWEET POTATO STORAGE-ROT FUNGI WHEN GROWN ON A NUTRIENT SOLUTION

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INTRODUCTION

Respiration in plants has been a favorite subject for botanical research for many years. Most of the earlier work was done with chlorophyll green plants, or parts of plants, some of the classical studies having been made upon seeds. The results of these researches have given us a better insight into the metalbolism of flowering plants, with some facts regarding the utilization of carbohydrates in respiration. Hasselbring and Hawkins (*9*)[1] in studying the respiration of the roots of sweet potatoes found that the reducing sugars were the immediate source of respiratory material. The cane sugar remained relatively stable when once formed and did not appear to be readily utilized so long as starch and other carbohydrates were present in abundance.

The results of investigations reported by the writers (*23*) show that the fungi responsible for most of the decay of sweet potatoes—namely *Fusarium acuminatum* E. and E. emend. Wollenw., *Sclerotium bataticola* Taub., *Diplodia tubericola* (E. and E.) Taub., *Penicillium* sp., *Mucor racemosus* Fes., *Botrytis cinerea* Pers., *Rhizopus tritici* Saito, and *Sphaeronema fimbriatum* (E. and H.) Sacc.—with the exception of the last-named organism, can utilize glucose as a source of carbon. Not only were these fungi able to use glucose in various amounts, but they also produced certain changes in the hydrogen-ion and osmotic concentrations of the culture medium. Some of the sugar was used in producing mycelium and in supplying energy for carrying on the vital processes of the organisms, although no doubt a part of it was utilized for other purposes.

The investigations reported in this paper were designed to throw further light (1) upon the question of the availability of glucose as a source of carbon for these same fungi, *Sphaeronema fimbriatum* excepted, and (2) upon the amount of the carbohydrate used in respiration as measured by the amount of carbon dioxid (CO_2) given off.

METHODS

In these investigations the fungi studied were grown in Erlenmeyer flasks on a liquid synthetic medium. The apparatus was set up in duplicate,

[1] Reference is made by number (italic) to "Literature cited," p. 225–226.

Journal of Agricultural Research, Washington, D. C.
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Vol. XXI, No. 4
May 16, 1921
Key No. G–230

and parallel experiments were run in most cases. The culture flasks were placed in an incubator held at a constant temperature, and CO_2-free air was pulled through by means of a Richard air pump. The air was passed through three bottles containing pumice stone and concentrated potassium hydroxid (KOH) and one bottle containing concentrated barium hydroxid ($Ba(OH)_2$), the latter being used principally as an indicator to insure that the air was entirely freed of CO_2. The air was finally washed by being pulled through CO_2-free distilled water, from which it was drawn into the culture flasks. The culture flasks and trap bottles for freeing the air of CO_2 and for washing it were placed in an incubator maintained at a temperature of 29° C. From the culture flasks the air was drawn out of the incubator through glass tubing. It was then freed of CO_2 by being drawn through a series of cylinders containing a saturated solution of $Ba(OH)_2$. Fresh air, though not in sufficient amount to interfere with the temperature control, was admitted to the incubator through a small hole about 1 cm. in diameter.

The cultures were grown in 1-liter Jena Erlenmeyer flasks stoppered with 2-holed rubber corks, through each of which were passed two glass tubes which served for the exchange of air. The tubes which admitted the air into the flasks extended to within about 1 inch of the surface of the medium, while the exit tubes barely projected through the corks.

CULTURE MEDIUM

A modification of Czapek's nutrient solution, in which ammonium nitrate (NH_4NO_3) was substituted for sodium nitrate ($NaNO_3$), was used as a substratum. Baker's C. P. dextrose in approximately 10 per cent strength was supplied as a source of carbon. The culture medium was prepared according to a method fully discussed in an article by Weimer and Harter (*23*), to which the reader is referred for complete details.

PREPARATION OF AN EXPERIMENT

Three 1-liter flasks, one to serve as a control, were prepared to contain 300 cc. of the culture medium. After being plugged with cotton they were sterilized by steaming for 20 minutes on three consecutive days. Just before inoculation the cotton plug was replaced by the rubber stopper with glass tubes attached, and the whole was resteamed for 20 minutes. To allow for the expansion on heating and to prevent contamination on cooling a glass tube of 3-mm. bore, about 8 cm. long, with a small bulb about 1 cm. in diameter midway between the two ends, was connected with each of the tubes passing through the cork by means of rubber tubing. The bulb was packed with cotton, which while permitting the passage of air served to filter out all contaminating material. The cotton was left in the bulb throughout the experiment.

Inoculations were made with a loop of a heavy spore suspension in distilled water or with a bit of mycelium from a young and vigorous cul-

ture growing on Irish potato cylinders or stems of *Melilotus alba* Desr. Before the culture flasks were inserted into the apparatus, air was pulled through the series of trap bottles and cylinders for about one hour to make sure (1) that all of the CO_2 was being removed from the incoming air and (2) that there was no leak through the connections.

At the termination of each experiment the mycelium was filtered into a tared alundum crucible washed with distilled water and dried to constant weight in a vacuum oven at 60° C. The percentage of glucose present in the control as well as that remaining in the two culture flasks was determined by a Fric saccharimeter, from which the total amount reduced was calculated. The culture and control flasks were weighed at the beginning and at the close of the experiment, and from these data corrections were made for loss of water due to evaporation or other causes.

DETERMINATION OF CO_2

The fungi differed markedly in the rapidity at which they grew in the culture solution. Some of the organisms, as *Rhizopus tritici*, for example, grew rapidly from the outset, and CO_2 was evolved in from two to three days. Some of the other fungi, on the other hand, grew slowly and gave off no CO_2 for a week or more. As soon as a precipitate appeared in the $Ba(OH)_2$ solution the CO_2 was determined for each 24-hour period thereafter to the end of the experiment.

In the determination of the CO_2 evolved the excess $Ba(OH)_2$ was neutralized by the addition of hydrochloric acid (HCl), with the use of thymol blue (thymol sulphonphthalein) as an indicator. The precipitate ($BaCO_3$) was then dissolved by adding an excess of *N*/1 HCl. After the total volume of the solution had been determined, an aliquot portion (usually 25 cc.) was titrated against *N*/10 sodium hydroxid (NaOH) with brom phenol blue (tetra bromo phenol sulphonphthalein) used as the indicator. The number of cubic centimeters of *N*/10 NaOH used to neutralize 25 cc. of the solution was multiplied by the total volume of solution, which gives the equivalent of the excess acid. The excess acid was then converted into its equivalent of *N*/1 HCl, and this amount was deducted from the total number of cubic centimeters of HCl required to dissolve the precipitate. The figure thus obtained was multiplied by the factor 0.022, the equivalent in CO_2 of 1 cc. of *N*/1 HCl.

In the preliminary experiments the two indicators, phenolphthalein and methyl orange, usually used in titrations of this nature were tried, but neither gave a satisfactory end point. Scales (*18*) experienced like difficulties in titrations of a similar kind and found that thymol blue and brom phenol blue both had a very sharp end point. Thymol blue in the presence of $Ba(OH)_2$ and $BaCO_3$ gives a brilliant blue color, which changes to a muddy green at the neutral point, to a lemon-yellow in a slight excess of acid, and to a pink in a strong acid solution. Upon the

addition of brom phenol blue the solution changes to a deep blue color and when slightly acid to a lemon-yellow.

EXPERIMENTAL DATA

RATE OF RESPIRATION

The rate of respiration of the different fungi expressed in CO_2 production is shown by the curves in figure 1, where the abscissae represent days and the ordinates the amount of CO_2 produced daily in grams.

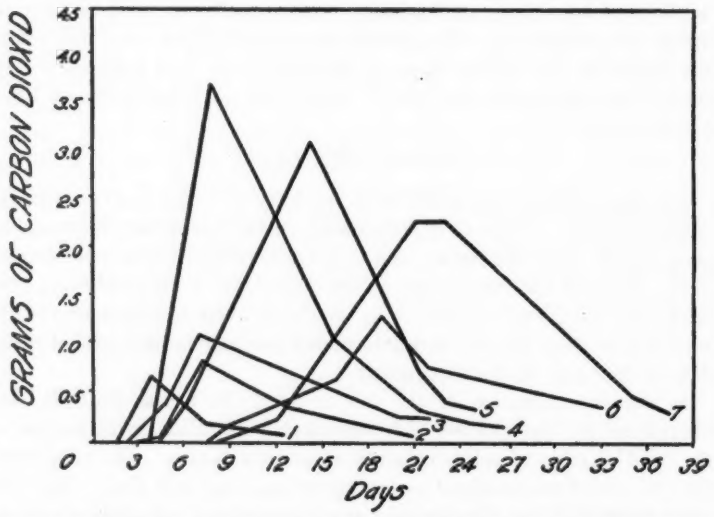

FIG. 1.—Rate of respiration of various fungi: 1, *Rhizopus tritici;* 2, *Diplodia tubericola;* 3, *Mucor racemosus;* 4, *Penicillium* sp.; 5, *Sclerotium bataticola;* 6, *Fusarium acuminatum;* 7, *Botrytis cinerea.*

The figures used in plotting these curves are in most cases the averages of two parallel tests. One familiar with the physiology and growth of fungi is aware that the same nutrient medium, the same temperature, and other environmental factors do not meet the requirements of all fungi equally well. In order to make the results comparable, a uniform standard of conditions for all the organisms was established as nearly as possible. They were all grown in an incubator maintained at a constant temperature of 29° C. in the dark.

An inspection of the curves shows that three organisms—namely *Penicillium* sp., *Botrytis cinerea,* and *Sclerotium bataticola* produced a quantity of CO_2 in excess of 2 gm. in a single day, while the four remaining organisms produce a relatively small amount. The data show also that those organisms which grew rapidly produced a comparatively small amount of CO_2, reaching their maximum in a short time after the culture flasks were inoculated and declining steadily thereafter. *Rhizopus*

tritici illustrates this point. On the second day it produced a measurable amount of CO_2, the quantity steadily increasing up to the fourth day. Thereafter it gradually decreased and practically ceased entirely by the twelfth day. *Penicellium* sp., *B. cinerea*, and *S. bataticola*, on the other hand, grew slowly and did not give off a measurable quantity of CO_2 for several days following inoculation. The evolution of CO_2 rapidly increased thereafter and continued a considerable number of days before it ceased entirely.

Except with *Botrytis cinerea*, the day of maximum production of CO_2 was followed by a sharp decline, which continued to the close of the experiment. *B. cinerea* differed from all the other organisms in that there was a period of two or three days when the maximum CO_2 production remained about constant. However, it is probable that if determinations had been made in less than 24-hour periods an apex in the curve would have been shown for this fungus.

DRY WEIGHT OF MYCELIUM

A direct comparison of the dry weight of the different organisms is not possible for reasons that can be well understood. As previously pointed out, the fungi were allowed to grow as long as a measurable quantity of CO_2 was given off. This varied from 11 to 30 days, so that some fungi had a much longer time to form mycelium. Furthermore, some fungi will produce a much larger quantity of dried material than others under identical conditions; in fact, it was shown by Kunstmann (*14*) that in duplicate cultures of the same fungus two and one-half times as much dried material might be produced in one flask as in another.

An examination of Table I and the curves in figure 1 will show that although the experiments with *Botrytis cinerea* ran for 38 days, *Penicillium* sp. produced about 11 per cent more dry weight in 10 days less time and that *Sclerotium bataticola* produced a greater dry weight in 25 days than *B. cinerea*, which grew 12 days longer. *Mucor racemosus* and *Rhizopus tritici* were grown 23 and 12 days and produced 1.13 and 0.94 gm. of dry weight, respectively. A comparison of *M. racemosus* with *S. bataticola* shows an even more striking difference, the ratio of dry weight being approximately 1 to 5.5.

TABLE I.—*Glucose reduced and dry weight and CO_2 formed by certain sweet potato storage-rot fungi, together with the numerical expression of the interrelationship existing between the glucose changed and the resulting products as well as between the products themselves*

Organism.	Solution.	Dry weight of mycelium produced (in grams).	Glucose remaining at the end of the experiment (in percentage by weight).	Amount of glucose reduced (in grams).	Total amount of CO_2 (in grams).
Fusarium acuminatum	Control	.....	9.2	.....	.....
	Inoculated	1.607	.55	27.510	15.524
Sclerotium bataticola	Control	.....	9.18	.....	.....
	Inoculated	6.068	.09	27.771	29.327
Diplodia tubericola	Control	.....	9.1	.....	.....
	Inoculated	1.972	6.35	7.628	6.066
Penicillium sp	Control	.....	9.2	.....	.....
	Inoculated	6.251	0	30.791	32.523
Mucor racemosus	Control	.....	9.1	.....	.....
	Inoculated	1.127	1.5	25.762	12.837
Botrytis cinerea	Control	.....	9.6	.....	.....
	Inoculated	5.377	.05	27.271	32.714
Rhizopus tritici	Control	.....	9.2	.....	.....
	Inoculated	.938	7.93	4.345	2.718

Organism.	Solution.	Grams of CO_2 given off per gram of glucose reduced.	Dry weight of mycelium (in grams produced by 1 gm. of glucose).	Coefficient of respiration: sugar (in grams reduced per gram CO_2 formed).	Economical coefficient: sugar used (in grams to produce 1 gm. dry weight).	Respiratory quotient: dry weight (in grams formed per gram CO_2 given off).
Fusarium acuminatum	Control	.....	.....	.....	.....	.....
	Inoculated	0.567	0.058	1.77	17.11	0.103
Sclerotium bataticola	Control	.....	.....	.....	.....	.....
	Inoculated	1.056	.218	.95	4.58	.207
Diplodia tubericola	Control	.....	.....	.....	.....	.....
	Inoculated	.795	.258	1.26	3.86	.325
Penicillium sp	Control	.....	.....	.....	.....	.....
	Inoculated	1.056	.203	.95	4.93	.192
Mucor racemosus	Control	.....	.....	.....	.....	.....
	Inoculated	.498	.044	2.01	22.86	.088
Botrytis cinerea	Control	.....	.....	.....	.....	.....
	Inoculated	1.200	.197	.83	5.07	.164
Rhizopus tritici	Control	.....	.....	.....	.....	.....
	Inoculated	.625	.216	1.59	4.63	.345

UNCHANGED GLUCOSE REMAINING IN THE SOLUTION

With the exception of *Diplodia tubericola* and *Rhizopus tritici* the fungi either utilized directly practically all the glucose originally present in the solution or converted it into other substances (Table I, columns 4 and 5), the former utilizing about 2.8 and the latter 1.3 per cent. The percentage of glucose consumed should be carefully compared with the dry weight of mycelium shown in column 3. *Fusarium acuminatum* and *Sclerotium bataticola* utilized all but 0.55 and 0.09 per cent, respectively, of the glucose and produced 1.61 and 6.1 gm. of mycelium. In

other words, *S. bataticola* made nearly four times as much dry weight in nine days less time by the utilization of practically the same amount of sugar as *F. acuminatum*. *Penicillium* sp., on the other hand, utilized all the glucose and formed 6.25 gm. of mycelium, while *Mucor racemosus* consumed 25.8 gm. of sugar in making 1.13 gm. of dry weight. A study of the figures shows that there is no uniformity among the fungi as regards the glucose utilized and dry weight produced, even if an account is taken of the length of time they were grown.

CO_2 PRODUCTION

In column 6 is shown the total amount of CO_2 produced by each fungus during the time of the experiment. A comparison of the total CO_2 given off with the dry weight produced and sugar utilized will not be necessary in all cases, but attention will be called to a few outstanding examples. *Sclerotium bataticola* gave off nearly twice as much CO_2 as *Fusarium acuminatum* and yielded nearly four times as much dry weight, while *Botrytis cinerea* and *Rhizopus tritici* produced 32.71 and 2.72 gm. of CO_2, respectively, and formed 5.38 and 0.94 gm. of dry weight. Here also a résumé of the figures shows that there is little or no uniformity in the relation of CO_2 evolved to the glucose consumed and dry weight produced.

RELATION OF CO_2 GIVEN OFF TO GLUCOSE REDUCED

The amount of CO_2 given off in grams for every gram of sugar reduced is shown in column 7. It will be seen that for *Sclerotium bataticola*, *Penicillium* sp., and *Botrytis cinerea* more than 1 gm. of CO_2 is produced for each gram of sugar consumed. In all the other cases it is less than unity. It should be noted that these are the three fungi which produced the largest amount of dry weight and are among those which consumed the most glucose. If extremes are contrasted it will be seen that *B. cinerea* and *Mucor racemosus* produce approximately 1.2 and 0.5 gm. of CO_2, respectively, for each gram of sugar utilized. While some reasons for these differences will be suggested later, attention should be called to the fact that *B. cinerea* continued to give off an appreciable amount of CO_2 some days longer than *M. racemosus*. Botrytis, Penicillium, and Sclerotium consumed all or practically all the sugar, so that it is not unlikely that other compounds were formed from the sugar which may have been utilized as a source of carbon.

RELATION OF DRY WEIGHT TO GLUCOSE CONSUMED

An examination of column 8 shows that the fungi differed greatly in the dry weight formed from 1 gm. of sugar consumed. *Fusarium acuminatum* and *Mucor racemosus* stand out as conspicuous examples of fungi which produce a small amount of mycelium per gram of sugar used, this

being only 0.058 and 0.044 gm., respectively. Contrasted to these, *Diplodia tubericola* formed the most, namely, 0.258 gm., while *Sclerotium bataticola* and *Rhizopus tritici* formed 0.218 and 0.216 gm., respectively.

COEFFICIENT OF RESPIRATION

The number of grams of sugar reduced per gram of CO_2 evolved is shown in column 9. The differences in the case of several of the fungi are very large. *Mucor racemosus* is the most extravagant and *Botrytis cinerea* the most economical user of sugar when the comparison is made on the basis of CO_2 produced, but it may be otherwise when the dry weight of fungus substance alone is considered. Four of the fungi studied require more and three less than 1 gm. of sugar to make 1 gm. of CO_2. Kunstmann (*14*) in working with *Aspergillus niger* v. Tieg. in a 5 per cent solution of cane sugar found a considerable variation in the results obtained from different experiments. In all cases, however, more than 1 gm. of sugar was required to form 1 gm. of CO_2, the variation ranging from 1.05 to 1.98. He further showed that a greater growth of the fungus was accompanied with an increase in respiration, as would be expected, and that the respiration became more rapid as the temperature rose. He showed, furthermore, that the concentration of the cane sugar in the nutrient solution influenced the rate of respiration, it being about 1.5 times as rapid in a 30 as in a 5 per cent solution. In a solution made slightly acid with phosphoric acid (P_2O_5) the quantity of CO_2 evolved by a unit weight of growth was considerably less than in those which remained alkaline.

It is evident that there are a number of factors which might have some influence on the evolution of CO_2 in experiments with a number of fungi. It is not possible to find an environment which would be the optimum for all of them. If one considers the concentration of the solution alone with respect to the source of carbon, which Kunstmann showed to influence respiration, it is clear that a concentration which might be considered optimum for one organism might not necessarily be so for another. In spite of the great ability of fungi to adapt themselves to solutions of high osmotic concentration, it is a well-known fact that some organisms can tolerate a more concentrated solution than others. The Penicillia, for example, grow in a sugar solution of high concentration. The writers found that *Botrytis cinerea* and *Penicillium* sp. grew equally well in a concentration of 38 per cent and 58 per cent, respectively. Bearing in mind the influence the concentration has on respiration one may readily conceive how the quantity of sugar present, which in these experiments was alike for all the organisms, might work to the advantage or disadvantage of the different fungi, at least so far as the amount of sugar required to produce 1 gm. of CO_2 is concerned.

Other factors which conceivably might influence the results are (1) acidity of the solution, (2) spore sowing, (3) light, (4) temperature. As

regards the first of these, it is a well-recognized fact that most fungi prefer a slightly acid medium, and in some cases if the solution is not acid they make it so. Since this subject is to be discussed more in detail in another connection, suffice it to say here that, as with the concentration, it is probable that the degree of acidity of the original solution was not optimum for all the organisms. Kunstmann considered the question of mass inoculation of the culture by spores and found that it exercised very little influence on the rate of respiration. There is at present some difference of opinion as to the influence of light on the respiration of fungi. Kreusler (*13*) and Wehmer (*22*) deny the influence of light on growth and respiration, while Bonnier and Mangin (*1*) and Elfving (*6*) ascribe a retarding effect and Ziegenbein (*24*) a favorable influence on some flowering plants. Probably all fungi will grow in the dark. Janssens and Mertens (*10*) found that the red Torula is influenced by light and behaves like green plants, respiration being apparently more pronounced in the light than in the dark. The writers have also found that certain organisms fruit abundantly when exposed to moderately strong light but remain sterile if grown in the dark. In the writers' present experiments the fungi were exposed to light for only a short period of time each day, when the incubator was opened to adjust the apparatus or to examine the cultures.

The temperature of 29° C. at which the organisms were grown probably does not represent the optimum for many, if it does for any of these fungi. The results of many investigators have shown that there is a wide range of optimum temperatures between different organisms, some thriving as well at 37° as others at 25°. In fact, there is considerable variation between species of the same genus. Brooks and Cooley (*3*) showed that the apple-rot fungi vary in their temperature requirements, and Edson and Shapovalov (*5*) showed that certain potato-rot and wilt-producing organisms of the potato had different optimum and maximum temperatures for growth. The writers found, for example, that *Rhizopus tritici* grows better at 35° than at 29° and will make a good growth at 40° or above. Some intermediate temperature, therefore, had to be employed which would permit all the organisms to make a good growth. Furthermore, the fact must not be overlooked that the rate of CO_2 production is more or less correlated with the temperature at which the fungus is grown. Ziegenbein (*24*) found that the optimum temperature for respiration of different flowering plants varied from 35° to 45°, and Kunstmann (*14*) and Stoklasa (*19*) report a similar variation for fungi and beetroot, respectively. According to these investigators the maximum rate of respiration correlates closely with the maximum temperature for growth. When the maximum temperature for growth is reached, the rate of respiration declines rapidly.

ECONOMIC COEFFICIENT

Pfeffer (*17*) and Kunstmann (*14*) have termed the numerical relation between the sugar consumed and the dry weight of the substance formed the "economic coefficient." According to Jost (*11*) the theoretical minimum value of the coefficient is about ½, but in reality it has been found to be higher than unity. Kunstmann found in working principally with *Aspergillus niger* that it varied from 1.13 to 3.88 in parallel experiments in a 5 per cent solution of cane sugar. Ono (*16*), on the other hand, obtained values as high as 6.1.

The results obtained by the writers are shown in column 10 of Table I. It will be seen that the economic values for *Fusarium acuminatum* and *Mucor racemosus* are very much higher than any of those given by Kunstmann and Ono, being 17.11 and 22.86, respectively. The five other organisms are fairly consistent in the amount of sugar required to produce 1 gm. of dry substance, all of which, however, are equal to or higher than the maximum given by Kunstmann. In view of these facts, it is quite evident that fungi in general can in no sense be regarded as economic users of sugar. In none of the writers' experiments or in those of Kunstmann and Ono has the minimum value of the economic coefficient fallen below unity.

From the data at hand it is evident that no sweeping generalizations can be made for all fungi. Ono showed that the addition of a small amount of zinc sulphate reduced the "economic coefficient," and Jost points out that the coefficient increases with the progressive development of the fungus and with an elevation of the temperature. Since the progressive development of the fungus influences the coefficient, the element of time would have an important bearing on the results. Although all of these experiments were carried out at the same temperature (29°C.), no doubt the fungi studied did not respond to heat in a similar manner.

RESPIRATORY QUOTIENT

The dry weight of fungus material produced for each gram of CO_2 given off is in all cases considerably less than unity (Table I, column 11). To contrast the extremes, *Rhizopus tritici* formed about four times as much dry material as *Mucor racemosus*. Kunstmann in all his experiments obtained a much higher numerical value of the respiratory quotient with *Aspergillus niger* in a 5 per cent cane sugar solution. In a few cases considerably more than 1 gm. of dry weight was produced for each gram of CO_2 given off. Kunstmann's results are not in every respect comparable, since he used different temperatures in different experiments. The higher temperatures for the most part appeared to lower the value of the "respiratory quotient." However, it may be concluded that in general under experimental conditions considerably more than 1 gm. of CO_2 is given off for each gram of fungus material formed.

These variations are more or less dependent upon the temperature at which they are carried out and the length of time the experiment has run.

DISCUSSION OF RESULTS

PRODUCTS OF FERMENTATION

ALCOHOL.—It is well known that fungi often produce alcohol and various organic acids as fermentation products during respiration. If the oxidation of the sugar was complete, CO_2 and water only would be produced, but results obtained by various workers have shown that other substances are often formed. An extensive literature is extant on the production of alcohol by different fungi in culture, but no attempt will be made to review all or any considerable part of it. Suffice it to say that the results of Brefeld (*2*), Fitz (*7*), and Hansen (*8*) with different species of Mucors, Aspergillus, Rhizopus, and Penicillium, and more recenty Kostytschew (*12*) with *Aspergillus niger* show that alcohol production by fungi is not uncommon. The amount of alcohol produced by the different organisms, according to the authors cited, differs with the medium used, the temperature, and the length of time the organism was grown. *Mucor racemosus* was found by Hansen to produce as much as 7 per cent by volume in 12 months at room temperature, and Fitz showed that *M. mucedo* Bref. would form 0.8 per cent alcohol by weight in 7 weeks at a temperature of 30°C.

Obviously it is not possible to determine with any degree of accuracy by present chemical methods just what a fungus does in a solution as complex as Czapek's nutrient medium. The writers' experiments showed that alcohol was produced to a limited extent by four of the fungi studied—namely, *Fusarium acuminatum*, *Rhizopus tritici*, *Diplodia tubericola*, and *Mucor racemosus*. *R. nigricans* and *M. racemosus* were shown to be alcohol producers by other investigators, the former to a very limited extent, and the latter in considerable quantity. So far as the writers are aware, no one has reported the production of alcohol by *F. acuminatum*, *R. tritici*, or *D. tubericola*. If alcohol was produced by the other fungi it was either utilized by the fungus or formed in such a limited amount that it could not be detected by the method employed. The results from which the conclusions were drawn, although not unqualified proof, were determined by the following method: One hundred cubic centimeters of the solution were neutralized with magnesium carbonate ($MgCO_3$). Fifty cubic centimeters were then distilled off, and from this a 25-cc. fraction was taken. The iodoform test was applied to the last distillate. A positive test was obtained in most cases only upon warming. The second distillate in all cases, when the iodoform test indicated the presence of alcohol, had a lower specific gravity than water. The same tests were carried out with the control solutions with negative results.

In no case was enough CO_2 produced to account for all the glucose used up, so that the question of what became of the remainder of the glucose naturally suggested itself. Attention already has been called to the fact that many fungi produce alcohol in nutrient solutions. According to Jost (*11*) a 10 per cent solution of alcohol is usually injurious to fungi, while a 2 to 4 per cent is usually nutritive. *Sclerotium batatícola*, *Penicillium* sp., and *Botrytis cinerea* did not produce alcohol according to the iodoform test, and yet they used up all or nearly all the glucose. While the writers have no proof to offer, it is possible that alcohol was formed by these organisms which was utilized by the fungi as a source of carbon.

Positive iodoform tests were obtained for *Rhizopus tritici*, *Diplodia tubericola*, *Fusarium acuminatum*, and *Mucor racemosus*, while *Botrytis cinerea*, *Sclerotium batatícola*, and *Penicillium* sp. gave negative results. It will be seen that the last three organisms produced more than 1 gm. of CO_2 for each gram of glucose used, while the first group gave off considerably less. The distillate from the solution on which *M. racemosus* grew gave an especially heavy precipitate of iodoform crystals.

Under the conditions of the experiment *Rhizopus tritici* and *Diplodia tubericola* produced only a trace of alcohol. A separate experiment was conducted to test further the ability of the former organism to form alcohol. Flasks were prepared in triplicate, with the same medium as in the previous experiments and 10 per cent glucose as the source of carbon. All three of the flasks were inoculated, two being stoppered with rubber corks and one with a cotton plug. A normal growth of mycelium took place in the flask stoppered with cotton, but in the other two fiasks the mycelium was abnormal in appearance, less luxuriant, and mostly submerged. Distillations were made from all the solutions, and as indicated by the iodoform test an abundance of alcohol was formed in the flasks stoppered with corks, while only a mere trace could be detected in the flask plugged with cotton. It would seem then that *R. tritici* will produce alcohol much more readily when growing on the medium used in the experiments recorded above when a reduced supply of oxygen is available and intermolecular respiration is thereby induced. The concentration of the CO_2 would be increased also under these conditions. In the respiration experiment, as indicated by the amount of CO_2 produced per gram of sugar used, it is possible that considerable alcohol was formed and possibly a part of it was carried off by the air current continually passing through the flask. However, some of the sugar may have gone to form other organic substances.

ORGANIC ACIDS.—The nutrient solution with which these experiments were conducted was slightly acid at the beginning. In a paper by Weimer and Harter (*23*) it was shown that all of these same organisms when growing in a 10 per cent glucose solution increase the P_H value of the solution and some of them to a considerable extent. It has been hither-

to shown by many investigators that fungi frequently increase the acidity of the substratum. Wehmer's (*20*, *21*) work in this direction is significant. He found that *Aspergillus niger* rendered the solution at first acid. The acidity (oxalic acid) gradually increased to a maximum and declined once more during the next few weeks to zero, the solution finally becoming alkaline. The fungus was found to decompose free oxalic acid at the higher temperatures. He likewise showed that the amount of acid formed was not necessarily associated with the quantity of fungous growth produced. The acid is found only when the substratum gives no acid reaction and when the organism is cultivated in sugar, proteid, glycerin, oil, and salts of organic acids. Wehmer also found that *Citromyces glaber* Wehm. can utilize the citric acid which it has produced. Furthermore, although this organism will tolerate a concentration of 20 per cent citric acid, it only produces enough to render the substratum 4 per cent acid. In a discussion of his results he calls attention to the fact that the only acids formed by Aspergillus or Penicillium in notable quantities are oxalic and citric. *Botrytis cinerea* and *Rhizopus nigricans* and some other fungi produce oxalic acid only in traces and only in a nearly neutral medium. An abundant carbohydrate supply and calcium salts, such as calcium phosphate or carbonate, favor its production. Kunstmann (*14*) found that oxalic acid was produced in all the media used by him but that at the end of the experiment it never exceeded 0.05 gm. in 100 cc. of solution. Molliard (*15*), however, found that *Sterigmatocystis nigra* v. Tieg. produced both citric and oxalic acid, together or alone in the medium in which saccharose was used as the source of carbon. Citric acid was more abundantly produced than oxalic, but both increased gradually up to the end of the experiment. The acid production of a large number of species of Penicillium was studied by Currie and Thom (*4*), who found that it was formed in varying amounts by all of them and in a large quantity by one species in particular, *Penicillium oxalicum* Currie and Thom. According to these investigators the oxalic acid produced is not an end product. It reaches its maximum in 8 to 12 days and then declines. The results of the writers' experiments show that in no case was enough CO_2 produced to account for all the sugar consumed. Undoubtedly some of the sugar was converted into other compounds, and in this connection alcohol, aldehydes, and organic acids suggest themselves as the most likely. The presence of alcohol was demonstrated in the culture solution in which the fungus grew in the case of four organisms and not in the controls. In another paper it was shown that these same organisms growing in the solution used in these experiments increased the hydrogen-ion concentration. It seems, therefore, that alcohol or acids or both may have been produced in the solutions.

SUMMARY

(1) The following fungi can utilize glucose as a source of carbon: *Fusarium acuminatum*, *Sclerotium bataticola*, *Diplodia tubericola*, *Penicillium* sp., *Mucor racemosus*, *Botrytis cinerea*, and *Rhizopus tritici*.

(2) *Penicillium* sp., *Botrytis cinerea*, and *Sclerotium bataticola* produced a maximum of a little more than 2 gm. of CO_2 in a single day. The other fungi formed a relatively small amount. The organisms which grew rapidly produced a comparatively small amount of CO_2 and reached their maximum in a short time after the culture flasks were inoculated. In all cases the respiration was measured as long as CO_2 was given off in any measureable quantity.

(3) The three fungi, *Penicillium* sp., *Botrytis cinerea*, and *Sclerotium bataticola*, which grew slowly, produced a relatively large amount of dry material and consumed all or nearly all of the glucose. The reverse is true of the other organisms.

(4) The quantity of CO_2 evolved does not necessarily correlate with the amount of dry material formed or with the amount of glucose reduced. Some organisms (*Mucor racemosus* and *Fusarium acuminatum*) which produced a comparatively small quantity of dry material reduced a large amount of sugar.

(5) Three organisms evolved more than 1 gm. of CO_2, the others considerably less, for each gram of glucose reduced.

(6) The dry weight of material per gram oi glucose consumed is in all cases considerably less than unity.

(7) The "coefficient of respiration" varies from 0.83 to 2.01, the "economic coefficient" from 3.86 to 22.86. The "economic coefficients" of *Fusarium acuminatum* and *Mucor racemosus* (17.11 and 22,86, respectively) are several times higher than that of any of the other fungi studied. They are also higher than the values given by other investigators.

(8) The quantity of CO_2 evolved was not equivalent to the theoretical amount that might have been produced from the sugar consumed. Other investigators have shown that alcohol is formed by *Mucor racemosus*, but the writers have demonstrated for the first time that it is produced by *Fusarium acuminatum*, *Rhizopus tritici*, and *Diplodia tubericola*. It was previously shown by the writers that these same organisms when growing in a 10 per cent solution of glucose increase the acidity of the solution. It is therefore probable that some of the glucose was utilized in the production of alcohol and acids.

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HERITABLE VARIATIONS IN AN APPARENTLY UNIFORM VARIETY OF COTTON

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The Pima variety of American Egyptian cotton, which originated with a single plant selection in 1910 and of which approximately 100,000 bales were produced in 1920, is probably the least variable of commercially grown cottons. So great, indeed, is the apparent uniformity that one might question the utility of further selection within the variety and of roguing seed-increase fields which are not exposed to accidental contamination. The justification of these procedures rests upon proof that heritable variations are not infrequent, and the object of this paper is to examine the evidence of their occurrence.

It is possible to measure the results of selection in the Pima variety because, fortunately, some of the seed produced by the parent plant of 1910 had been preserved. When plants grown from this seed in 1919 were compared with the present commercial stock and with a line-bred strain it was found that although there has been no definite change of type the modes for certain characters have been altered by selection. Additional evidence of the occurrence of slight but heritable variations has been obtained in the course of the breeding and roguing work. A much wider variation, outside the normal range of the Pima variety but not attributable to recent hybridization, has also proved to be heritable.

EARLY HISTORY OF THE PIMA VARIETY

The Pima variety originated with a plant discovered at Sacaton, Ariz., in 1910, in a field of the Yuma variety. The Yuma variety likewise originated with a single plant discovered at Yuma, Ariz., in 1907 in a stock of the Mit Afifi variety which had been introduced from Egypt several years previously. The parent plants of both the Yuma and Pima varieties were so different from the stocks in which they appeared and their progenies expressed the new characters so uniformly that their origin was attributed to mutation.[2]

[1] The writer is indebted to Messrs. Walter F. Gilpin and Robert L. Taylor for the photographs used in illustrating this paper.

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No flowers on the original Pima plant were bagged for self-pollination, for its valuable qualities were not appreciated until a progeny row was grown the following year. It was then realized that a new type had arisen which differed in many characters from the Yuma variety. The progeny was remarkably uniform in view of the fact that it was grown from open-pollinated seed. The immediate progeny of the first Pima plant was grown in 1911 adjacent to a planting of the older variety, but very little hybridization could have taken place, for as far as the record shows, all plants in the five progenies which were grown in 1912 from selections made in 1911 exhibited only the distinctive Pima characters. After 1912 the Pima progenies were isolated from other cottons, but controlled self-pollination was not practiced in the earlier breeding work with this variety.[1]

A single plant selection, No. 5, in the 1911 progeny of the original Pima plant was the parent of progeny No. 5 which was grown in 1912. From a single plant selection, No. 3, in this progeny has descended the strain, henceforth referred to as 5–3, of which approximately 250,000 acres were grown in Arizona and California in 1920.

COMPARISON OF THE PRESENT COMMERCIAL STOCK WITH THE IMMEDIATE PROGENY OF THE PARENT PLANT

A progeny of 32 plants was grown in 1919 from open-pollinated seed of the original Pima plant.[2] Plates 48 and 49 show the range of variation in shape and surface of the boll, each plant of the progeny being represented, with the exception of No. 5 which was accidentally omitted.[3] Plants 1 to 17 are represented in Plate 48 and plants 18 to 32 in Plate 49. Two of these plants showed characters outside the normal range of variation of the Pima variety as it now exists. The others appeared to be typical in all respects. One of the aberrant plants (Pl. 48, L) had bolls which were conspicuously shorter, broader at the base and near the apex, and less pointed than in typical Pima, and which resembled the bolls of a first-generation hybrid of the Pima and Gila varieties. The other plant, No. 28 (Pl. 49, K), had bolls that were atypical in being more slender and less well filled out, and in having a less conspicuous "shoulder" and a more deeply pitted surface—characters which distinguish the Yuma from the Pima variety.[4] All of the 28 individuals of a progeny grown in 1920 from selfed seed of plant No. 28 produced bolls which resembled the Yuma type in greater or less degree.

Plant No. 28 of 1919 resembled the Yuma variety also in the very small amount of fuzz on the seeds, having been, in fact, the smoothest-seeded

[1] Evidence has been obtained recently that in the Egyptian type of cotton most of the ovules are self-ertilized even when no precautions are taken to prevent cross-pollination.

[2] Although the seed was nearly 9 years old, the germination was excellent and the plants made a vigorous growth.

[3] The measurements indicate that plant No. 5 had bolls which were typical in shape.

[4] Typical bolls of the Yuma, Pima, and Gila varieties of Egyptian cotton are shown on Pl. 24, fig. 2 and 3, and Pl. 25, fig. 2, *in* KEARNEY, Thomas H., OP. CIT. 1914.

of the 32 plants of the Pima parent progeny.[1] The progeny grown in 1920 showed considerable variation in this character, but a majority of the plants had smoother seeds than the average Pima and a few individuals had almost naked seeds. It is therefore probable that plant No. 28 was a first-generation Pima × Yuma hybrid, the original Pima plant of 1910 having been surrounded by plants of the Yuma variety. It is surprising, under the circumstances, that in a progeny of 32 individuals only 1 showed recognizable Yuma characters.[2]

In order to measure the degree of change which might have taken place as a result of several years of selection in the Pima variety, the progeny of the parent plant grown in 1919 was compared with an equal

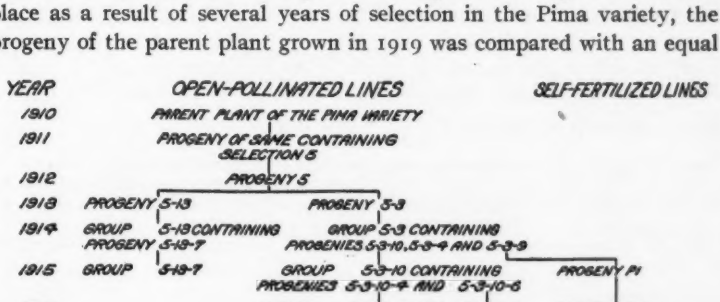


FIG. 1.—Pedigree of progenies, progeny groups, and strains of the Pima variety of American Egyptian cotton. Each progeny is from an individual plant selection of the preceding year. As here used, the term "progeny group" comprises the progenies of sister plants of the preceding year and the term "strain" comprises all lines derived from the same progeny group.

number of plants taken at random in the commercial stock of strain 5–3 and of two progenies (Y1A and Y2A) derived from the same strain by five generations of controlled self-fertilization. The ancestry of these selfed lines is indicated in figure 1. For the sake of brevity, the three populations will be referred to hereafter as "parent progeny," "5–3 bulk," and "5–3 selfed." All were grown under conditions of soil, irrigation, and spacing which were as nearly as practicable identical.

[1] The apparent dominance of a less fuzzy over a more fuzzy condition of the seed coat in this presumable first-generation hybrid is exceptional, since according to Balls (BALLS, W. Lawrence. SOME CYTOLOGICAL ASPECTS OF COTTON-BREEDING. *In* Amer. Breeders' Assoc. Rpt. 1908, v. 5, p. 18. 1909.) "little fuzz" is dominant over "no fuzz" and "entire fuzz" is dominant over "little or no fuzz". Similar relations are reported by McLendon (MCLENDON, C. A. MENDELIAN INHERITANCE IN COTTON HYBRIDS. Ga. Agr. Exp. Sta. Bul. 99, p. 169. 1912.). It has been the writer's experience that in hybrids between Egyptian varieties with partly fuzzy seeds and Upland varieties with completely fuzzy seeds the first generation has completely fuzzy seeds.

[2] Progenies comprising from 22 to 34 individuals were grown in 1920 from selfed seed of 5 plants of the "parent" progeny of 1919, and the leaf and boll characters listed in Table I were determined for all plants of each progeny. Comparison of the means of these progenies with the means of selfed and open-pollinated stocks of strain 5–3 showed no important differences, except that the mean boll diameter and boll index (diameter as a percentage of the length) of the progeny of plant 28 were significantly smaller than in any other progeny or strain of Pima on which these characters were measured in 1920. Comparison of the coefficients of variation for the several characters showed that progeny 28 was decidedly more variable than the other granddaughter progenies of the parent Pima plant, as would be expected if this progeny represented the second generation of a Pima × Yuma cross.

Measurements were made on one leaf and one boll of each plant,[1] endeavor having been made to select in each case a leaf and a boll which had developed normally and which appeared to be typical of the plant in question. Seed cotton was collected from 5 open-pollinated bolls on each plant, and the fiber on one seed from each boll was combed out and measured, the average of the 5 determinations being taken as the length of fiber for the plant in question. The seed cotton was then weighed and ginned, the weight of the fiber and the number of seeds in the sample were determined, and the lint index (weight in grams of fiber per 100 seeds) was calculated from the resulting data. Finally, the seeds were graded in respect to fuzziness by matching with a set of types representing the total range from the smoothest (grade 1) to the fuzziest (grade 7) condition of the seed coat. Table I shows for the 12 characters the means and coefficients of variation of the three stocks.

TABLE I.—*Means and coefficients of variation for 12 characters of a progeny of the parent plant of the Pima variety, of a "bulk" (continuously open-pollinated) population of strain 5–3, and of a line of the same strain which had been strictly self-fertilized during five generations*

Character.	Means.			Coefficients of variation.[1]		
	Parent.	Bulk.	Selfed.	Parent.	Bulk.	Selfed.
Leaf length (cm.)[2]	169±1.14	164±2.27	167±1.41	5.4±0.47	11.1±0.96	6.8±0.59
Leaf width (cm.)[3]	219±2.71	214±3.53	229±2.09	9.9± .86	13.2±1.15	7.3± .64
Leaf index [4]	129±1.37	130±1.35	137±1.11	8.5± .74	8.3± .72	6.5± .56
Leaf vein angle [5]	106±1.37	104±1.42	114±1.15	10.3± .90	11.0± .96	8.1± .70
Leaf lobe index [6]	268±2.06	261±1.64	284±2.38	6.1± .53	5.0± .44	6.8± .59
Leaf extension index [7]	19.5± .37	17.1± .46	20.5± .40	15.2±1.32	21.5±1.87	15.5±1.35
Boll length (mm.)	46.2± .24	47.1± .21	47.0± .23	4.4± .40	3.7± .31	4.1± .35
Boll diameter (mm.)[8]	27.3± .14	26.9± .08	27.9± .07	4.2± .35	2.6± .22	2.1± .18
Boll index [9]	59.2± .33	57.3± .28	59.5± .33	4.6± .39	4.0± .34	4.6± .39
Fiber length (mm.)	40.8± .19	44.6± .12	42.9± .12	3.8± .32	2.2± .19	2.4± .20
Lint index (gm.)	4.1± .06	5.6± .05	4.5± .06	13.2±1.11	7.9± .67	11.1± .94
Seed fuzziness	3.8± .17	4.1± .06	4.7± .11	1.4± .12	.5± .04	.9± .08

[1] In the case of seed fuzziness (graded) the standard deviations are compared. For all other characters the comparison is of coefficients of variation.
[2] Measured from base of midvein to tip of central lobe.
[3] Measured from tip to tip of the principal lateral lobes.
[4] Width as a percentage of the length, instead of length as a percentage of the width, the value used in the paper by Kearney and Wells, cited on p. 227. The new leaf index is the reciprocal of the old, multiplied by 10,000.
[5] Width (in degrees) of the angle formed by the principal lateral veins.
[6] Leaf length as a percentage of the distance from the base of the midvein to the bottom of the upper right hand sinus. A deeply lobed leaf has a high lobe index and vice versa.
[7] Length of the basal extension of the blade, below a line bisecting the base of the midvein at a right angle to the latter, as a percentage of the length of the leaf.
[8] At the point of greatest diameter.
[9] Maximum diameter as a percentage of the length, instead of length as a percentage of the diameter, the value used in the paper by Kearney and Wells, cited on p. 227. The new boll index is the reciprocal of the old, multiplied by 10,000.

The coefficients of variation of the parent progeny, as compared with those of the 5–3 bulk, were higher in 8 and lower in 4 of the 12 characters;

[1] Since only 30 plants of the "Pima parent" progeny had leaves in good condition for measurement, the leaf characters were determined on only 30 plants in the other two stocks. The boll, fiber, and seed characters were determind on all 32 plants of each stock. The leaf characters were measured on selfed progeny Y1A and the other characters on selfed progeny Y2A.

and as compared with the selfed line they were higher in 8 and lower in 3 characters. If we regard as significant only such differences as amount to at least three times the probable error, the parent progeny was significantly more variable than the bulk stock in 4 and significantly less variable in 1 of the 12 characters. As compared with the selfed population, the parent progeny was significantly more variable in 3 and significantly less variable in none of the characters.

The high degree of homozygosity apparently attained by the commercial stock as a result of simple selection without controlled selfing is indicated by the fact that five generations of controlled selfing has rendered the "selfed" line significantly less variable than the "bulk" stock in only 2 of the 12 characters, while in 1 character it is significantly more variable. No significant difference in variability is shown in respect to the 9 remaining characters.

FIBER AND SEED CHARACTERS OF THE PARENT PROGENY AND OF THE PRESENT COMMERCIAL STOCK

Especial interest attaches to the characters fiber length, lint index, and seed fuzziness because of their practical importance and because since the commencement of the breeding work with the Pima variety attention has been focused upon increasing the length and abundance of the fiber and obtaining a high degree of uniformity in these characters. An indication of what has been accomplished along these lines is afforded by the frequency distributions in Table II. The maximum and minimum length of fiber, abundance of fiber, and fuzziness of seeds of the parent progeny and of a corresponding number of plants of the commercial stock are represented in Plates 50, 51, and 52.

LENGTH OF FIBER.—It is evident from the data in Tables I and II that the immediate progeny of the parent plant of the Pima variety was much more variable in length of fiber and had a significantly lower mean for this character than a random sample of the present commercial stock of strain 5–3. This strain, as indicated in the pedigree shown in figure 1, was derived from a granddaughter of the original Pima plant, selected in 1912 largely on the basis of its superior length of fiber. The difference between the means of the "parent" progeny and of the bulk stock was 3.8 mm., hence more than ⅛ inch, and the difference was 17 times greater than its probable error. The selfed line of strain 5–3 was almost intermediate in length of fiber between the "parent progeny" and the "bulk," its mean differing from those of the other two populations by amounts equivalent to 9 and 10 times the probable error of the differences. The ancestors of this closely inbred line, beginning with plant P1 in 1914, had not been selected with reference to this character and would seem to have represented very nearly the minimum potentiality of strain 5–3 in respect to length of fiber. The coefficients of variation of the bulk and selfed populations do not differ significantly, but both were significantly less variable than the parent progeny.

TABLE II.—*Frequency distributions for the characters fiber length, lint index, and seed fuzziness of a progeny of the parent plant of the Pima variety, of a "bulk" (continuously open-pollinated) population of strain 5-3, and of a line of the same strain which was strictly self-fertilized during five generations, each population containing 32 plants*

FIBER LENGTH

	37 mm.	38 mm.	39 mm.	40 mm.	41 mm.	42 mm.	43 mm.	44 mm.	45 mm.	46 mm.	47 mm.
Parent progeny	1	3	2	6	11	5	1	2	1	....	....
5-3 bulk	....	....	....	....	....	....	5	9	13	4	1
5-3 selfed	....	....	....	2	2	4	15	6	3	....	....

LINT INDEX

	3.3 gm.	3.8 gm.	4.3 gm.	4.8 gm.	5.3 gm.	5.8 gm.	6.3 gm.	6.8 gm.	7.3 gm.
Parent progeny	4	10	13	2	3	.....	.....	.....	.....
5-3 bulk	.....	.....	.....	1	18	10	2	.....	1
5-3 selfed	.....	4	14	10	3	.....	1	.....	.....

SEED FUZZINESS

	Grade 1.	Grade 2.	Grade 3.	Grade 4.	Grade 5.	Grade 6.	Grade 7.
Parent progeny	1	5	7	8	8	3	.....
5-3 bulk	.....	.....	3	23	5	1	.....
5-3 selfed	.....	.....	1	13	13	3	2

QUALITY OF FIBER.—While no quantitative data on this point are available, it seemed evident in examining the samples of fiber from the individual plants of the three stocks that those of the "parent" progeny differed more among themselves in smoothness, luster, and color than did the plants of the "bulk" stock and of the selfed line. The smoothness or silkiness of the fiber also seemed to average higher in the two latter populations. Selection, therefore, would seem to have resulted in raising the mean of this character and in reducing its variability.

LINT INDEX.—The abundance of the fiber, as expressed by the mean weight of fiber borne by 100 seeds, averaged 36½ per cent greater in the "bulk," or commercial stock of the variety, than in the progeny of the original Pima plant. The difference between the means is very significant, amounting to 19 times its probable error. There had been also a significant reduction in variability, the coefficient of variation for lint index of the "bulk" stock being lower than that of the "parent" progeny by an amount equivalent to more than 4 times the probable error of the difference. Since increase in the abundance of fiber on the seed, like increase in length of fiber, has been one of the principal objectives of the breeding work with the Pima variety, it is interesting to

note this evidence of the progress attained. On the other hand, the selfed line, the immediate ancestors of which had not been selected with reference to this character, had a much lower mean and a higher, though perhaps not significantly higher, coefficient of variation for lint index than the bulk population of the 5–3 strain from which it was derived. In this character, also, five generations of controlled self-fertilization apparently had resulted in no further progress toward homozygosity.

FUZZINESS OF SEEDS.—In the earlier years of breeding work with the Pima variety no especial attention was given to this character. Consequently it is not surprising that the "bulk" stock, as compared with the parent progeny, showed no significant difference in the mean amount of fuzz on the seed. On the other hand, the variability had been significantly reduced, the standard deviation of the bulk stock being lower than that of the parent progeny by an amount equal to 7 times the probable error of the difference. The selfed line had a mean fuzziness which was significantly greater than that of both the "bulk" stock and the parent progeny, the difference in both cases being about five times its probable error. The selfed line was significantly more variable in seed fuzziness than the bulk stock but was significantly less variable than the parent progeny. It is evident that the selfed line had been unconsciously selected towards the upper limit of fuzziness represented in the immediate progeny of the parent plant of the Pima variety. Fuzziness is a marked disadvantage from a practical point of view, the separation of the fiber by the type of gin used for this very long staple cotton being much more easily effected with smooth than with fuzzy seeds. The frequency distribution of the parent progeny indicates that selection of a strain having smoother seeds than the present commercial stock of Pima is well within the limits of possibility.[1]

EVIDENCE OF THE OCCURRENCE OF HERITABLE VARIATIONS OBTAINED IN ROGUING

The Yuma variety of American Egyptian cotton was grown by Arizona farmers previous to the introduction of the Pima variety. In 1914, 1915, and 1916 fields of this cotton were rogued by agents of the United States Department of Agriculture, in cooperation with the Salt River Valley Egyptian Cotton Growers' Association, in order to supply seed to be increased for planting the commercial acreage. The total number of plants in the fields rogued during the three years was rather closely estimated as 2,290,000, of which (by actual count) 23,537, or 1.03 per cent, were removed as being off-type or otherwise undesirable. The work involved a rapid examination of every plant in the area rogued and thus afforded an excellent opportunity for judging the state of uniformity of the variety.

[1] This is true even if we leave out of consideration the single plant (No. 28) which falls into grade 1, this having been, in all probability, a first-generation Pima × Yuma hybrid.

A more careful examination was made at Sacaton in 1914 of 124 individuals taken at random in a field planted with ordinary "gin run" seed of Yuma cotton. Of these, 117 were classified as true Yuma, while 7, or 5.5 per cent of the total, were classified as distinctly off-type. Progenies grown in 1916 from selfed seed of the off-types and of plants regarded as typical Yuma proved to be, in most cases, remarkably uniform in the stem, leaf, and boll characters which had distinguished the parent individuals. A form which appeared to be analogous to the so-called "bull-stalk" variation in Sea Island cotton, characterized by a tall stem, long internodes of the axis and fruiting branches and rather stiff, semi-erect leaves, was so frequent as to be included within the normal range of variation of Yuma cotton, although this type also proved to be heritable.

Cooperative roguing was carried on during and after the substitution of the Pima for the Yuma variety. In 1916, 1917, 1919, and 1920 an estimated total of 3,600,000 plants were examined; and, by actual count, 10,221 plants, or 0.28 per cent of the total number, were removed from the seed-increase fields of the Pima variety. There was little variation in the amount of elimination from year to year, the figures having been 0.21 per cent in 1916, 0.42 per cent in 1917, 0.20 per cent in 1919, and 0.20 per cent in 1920. Although the roguing was much more rigorous than that practiced with the Yuma variety, the percentage of the total plants which were removed from the Pima fields in all four years was less than one-third as great. Most of the Pima "rogues" departed so little from the type that they would have been disregarded in roguing a field of the much more variable Yuma. A large majority of the plants removed were merely sickly or more or less sterile. Indisputable evidence was therefore obtained that the closer line-breeding which had been practiced from the beginning with the Pima variety had resulted in the development of a much more uniform stock.

The most distinct off-types in the Pima fields, in addition to a few very aberrant individuals of which the characters indicated hybridization with some other variety of cotton, were either tall, vigorous individuals having exceptionally long internodes (the "bull-stalk" type) or stiff, slender, grayish plants having semierect branches and leaves, the leaves being narrower, more deeply lobed, and with more incurved and undulate margins than in typical Pima. Both of these forms had their counterparts in the older Yuma variety, and it seems not improbable that they arise by recurrent mutation or recombination in all varieties of Egyptian cotton. Variation in the shape of the bolls was much less frequent and extreme than in the Yuma variety, but a few of the plants had shorter and less pointed, or longer and more taper pointed, bolls than in typical Pima.

In a bulk field of Pima cotton at Sacaton in 1917 certain individuals were selected as slightly off-type. Flowers were bagged on these plants,

and progenies were grown in 1919 from the resulting selfed seed. One of these progenies resembled the parent individual in having the surface of the boll more deeply pitted than in typical Pima. Another progeny showed a tendency to produce shorter and plumper bolls than the Pima type, while a third progeny showed the "bull-stalk" habit as well as a tendency to produce short, plump bolls. So far as this evidence goes it indicates the inheritance of slight variations in the Pima variety.

The recognition of these various types is always easiest in a well-grown field of cotton. If the plants are either too rank or too stunted, the variations are more or less masked. Certain soil conditions appear to favor the expression of a certain type. Thus, fields have been examined where the growth was subnormal and where all the plants showed a tendency to the stiff, grayish form which in most fields occurs scatteringly among plants of normal habit and color. On the other hand, where the growth is very rank, the "bull-stalk" type can scarcely be recognized.

EVIDENCE OF THE OCCURRENCE OF HERITABLE VARIATIONS OBTAINED IN LINE BREEDING

The records of the breeding work (selection) which has been carried on with the Pima variety since its origin in 1910 have been scanned for evidence of the occurrence of heritable variations. Unfortunately the stem, leaf, and boll characters had not been recorded systematically, but a few data based upon careful measurements are available. The type of plant which has been kept in mind as the ideal in breeding work with the Pima variety is shown in Plate 53. It is characterized by the absence of vegetative branches and by the development of good fruiting branches from near the base to the summit of the axis. The relationship of the various strains and progenies mentioned in the following pages is shown in figure 1.

In 1914 two groups (5–3 and 5–13), descended from sister plants in progeny No. 5 of 1912, were compared with respect to certain stem and branch characters and showed significant differences, as given in Table III.

TABLE III.—*Means for stem and branch characters in two lines of the Pima variety grown in 1914*

Character.	Group 5–3 (24 plants).	Group 5–13 (26 plants).	Differences between means.
Axis length (cm.)	178.0± 1.55	194.5± 2.11	16.5± 2.62
Axis internode number	40.5± .34	42.8± .43	2.3± .55
Axis internode length (cm.)[1]	44.0± .33	45.5± .25	1.5± .41
Limb index [2]	102.3±10.76	172.4±12.88	70.1±16.80

[1] Value for each plant obtained by dividing the length of the axis by the number of internodes.
[2] Aggregate length of vegetative branches divided by length of axis.

In 1915 the two strains, as represented by progeny groups 5–3–10 and 5–13–7, were further compared in regard to length of axis and development of the vegetative branches, with the results given in Table IV.

TABLE IV.—*Means for axis length and limb index in two lines of the Pima variety grown in 1915*

Character.	Group 5–3–10 (25 plants).	Group 5–13–7 (15 plants).	Differences between means.
Axis length (cm.)	148± 1.28	176± 2.96	28± 3.23
Limb index	115±12.30	144±12.56	29±17.60

The differences in the means for axis length and limb index are in the same direction as those for strains 5–3 and 5–13 in 1914, but the difference in limb index in 1915 was less than twice its probable error. This character, however, is extremely sensitive to environmental influence, and it is a fair assumption that the two strains are genetically different in respect to both the length of the axis and the development of the vegetative branches.

The records for 1914 permit comparison of still more closely related lines. In that year progenies were grown of several plants which had been selected in progeny 5–3 of 1913. One of these progenies (5–3–4) was noted as being very uniform and very distinct from the others, the plants having been stiff and open looking, with long fruiting branches, long internodes of the axis and fruiting branches, and rather stiff leaves which were deeply and narrowly lobed. This description recalls the "bull-stalk" type which was encountered frequently in roguing the seed-increase fields, except that the plants were not noticeably taller than in the other progenies. The differences in stem and branch characters, as compared with the progeny of a sister plant (5–3–10), are shown by the data in Table V.

TABLE V.—*Means for stem and branch characters of two progenies from sister plants, grown in 1914*

Character.	Progeny 5–3–4 (12 plants).	Progeny 5–3–10 (11 plants).	Differences in the means.
Axis length (cm.)	183.0±1.80	179.0±2.19	4.0±2.83
Axis internode number	38.0± .25	41.2± .39	3.2± .46
Axis internode length (cm.)	48.2± .35	43.5± .66	4.7± .75
Fruiting branch length (cm.)	63.2±1.19	56.5± .78	6.7±1.42

Progeny 5–3–4 had significantly fewer and significantly longer internodes of the axis and significantly longer fruiting branches than progeny 5–3–10, the differences having been, respectively, 6.9, 6.3, and 4.7 times their probable errors.

Two plants, selected in progeny 5–3–10 of 1914 and designated 5–3–10–4 and 5–3–10–6, gave rise to strains of which progenies were grown in 1916, 1917, and 1918. Measurements of the weight of seed cotton per boll, length of fiber, lint index (weight of fiber per 100 seeds), and fuzziness of the seeds were made in each year upon plants of each strain. Consistent and significant differences were found only in regard to the character seed fuzziness. The means of the grade numbers of the individual plants are given in Table VI.

TABLE VI.—*Means for seed fuzziness of two closely related lines of the Pima variety, grown in 1916, 1917, and 1918*

Group or strain.	1916		1917		1918	
	Number of plants.	Mean grade.	Number of plants.	Mean grade.	Number of plants.	Mean grade.
5–3–10–4	16	3.9±0.11	47	3.2±0.17	71	3.3±0.10
5–3–10–6	29	4.4± .11	35	3.8± .15	28	4.3± .10
Difference	.....	.5± .155	.....	.6± .226	.....	1.0± .142

Strain 5–3–10–6 is consistent in having had fuzzier seeds in each of the three years, although the difference in 1917 was scarcely significant. This case affords clear evidence of a slight but heritable difference between two lines which are very closely related, and of which the common ancestor (plant 5–3–10 of 1913) represented the third generation of linebred descent from the parent plant of the Pima variety.

INHERITANCE OF AN INCREASED NUMBER OF BOLL LOCKS

The boll of Pima cotton has usually 3 locks or carpels, although 4-lock bolls are borne by nearly all plants of this variety and bolls having 2 or 5 locks are met with occasionally. There is much individual variation in the proportion of 3-lock and 4-lock bolls. With a view to ascertaining whether such differences are heritable, several plants which had more or fewer 4-lock bolls than the average were selected in 1917 in an increase plot of a selected strain of Pima. Progenies of three such plants were grown in 1918 from seed produced by open-pollinated flowers. Table VII gives the percentages of bolls with 4 locks, all bolls on all plants in each progeny having been taken as one array in computing the percentage and its probable error.

TABLE VII.—*Percentages of 4-lock bolls in the progenies of individual plants selected in 1917*

Progeny.	Number of plants.	Percentage of 4-lock bolls.
No. 2 (from parent with few 4-lock bolls)	19	4.6±.031
No. 1 (from parent with many 4-lock bolls)	22	5.9±.33
No. 3 (from parent with many 4-lock bolls)	8	10.5±.67

The difference as between progenies 2 and 1 was barely three times its probable error, while as between progenies 2 and 3 it was more than six times its probable error.

Progenies were grown in 1919 from selfed seed of one plant in progeny No. 3 and of two plants in progeny No. 1 of 1918. In two of the progenies of 1919, flowers on a few plants were selfed and progenies from the resulting seed were grown in 1920. The percentages of 4-lock bolls on the plants of these progenies and on plants taken at random in a neighboring plot of bulk Pima, in both years, are given in Table VIII.

TABLE VIII.—*Percentages of 4-lock bolls in 1919 and 1920 in the progenies of plants selected for a high percentage of such bolls and in "bulk" plantings of Pima cotton*

Material.	1919		Material.	1920	
	Number of plants.	Percentage of 4-lock bolls.		Number of plants.	Percentage of 4-lock bolls.
4-lock progenies:			4-lock progenies:		
1–3	13	8.5±0.65	1–3–12	45	9.2±0.36
1–5	20	8.7± .54	3–2–4	39	22.0± .62
3–2	5	10.9± .87	3–2–5	42	23.3± .60
Combined	38	9.1± .37	Combined	126	17.2± .30
Bulk Pima	38	4.2± .27	Bulk Pima	50	5.3± .29

The descendants of plants which had been selected as showing a tendency to increased number of locks gave, in both years, significantly higher percentages of 4-lock bolls than plants taken at random in the general stock of the Pima variety. In 1919 the difference in favor of the 4-lock progenies was 10 ½ times and in 1920 it was 28 ½ times its probable error. In all three years (1918 to 1920) the descendants of selection No. 3 of 1917 gave a higher percentage of 4-lock bolls than the descendants of selection No. 1. These facts indicate clearly the heritable nature of this variation.

There can be little doubt, therefore, that a strain having a materially higher percentage of 4-lock bolls could be developed by selection, although the practical importance of such increase in the Pima variety would seem to be small. Determination by Mr. W. G. Wells in 1919 of the mean weight of seed cotton per boll in 100 3-lock and 100 4-lock bolls showed that the latter averaged only 10 per cent heavier than the former. Similar determinations by Mr. W. F. Gilpin in 1920 on 50 bolls of each lock number showed a difference of only 7 per cent in favor of the 4-lock bolls. Hence, even if it were possible to develop a strain of Pima cotton in which all the bolls would have 4 locks, the increase in yield of fiber and seed would not be likely to exceed 10 per cent.

INHERITANCE OF SPOTLESS OR FAINTLY SPOTTED PETALS

The variations which have been considered thus far are so slight that in the absence of evidence of their heritability, they would be regarded as mere fluctuations. Especial interest attaches to a variation which is outside the normal range of variation not only of the Pima variety but of the Egyptian type of cotton.

Inspection of the 4-lock progenies in 1919 revealed the surprising fact that in all plants in these three progenies there was either total absence or very faint development of the dark red spot near the base of the petal which is so marked a characteristic of the Pima variety and of Egyptian cotton in general. In this respect the 4-lock plants contrasted strikingly with all other Pima progenies in the same field as well as with the bulk stock of this variety grown in adjacent fields. Although there is appreciable variation in the size and in the intensity of color of the petal spot in Pima cotton, no such approach to complete absence of the spot had been observed hitherto except in plants which were obviously hybrids with Upland cotton.

The 38 plants of the three 4-lock progenies in 1919 were graded by Mr. W. G. Wells with respect to degree of development of the petal spot, grade 8 indicating the extreme development in typical Pima cotton and grade 0 indicating complete absence of the spot, as is the case in most varieties of American Upland cotton (*Gossypium hirsutum L.*). Two or three flowers were separately graded on each plant, and the averages of the resulting grade numbers were used in computing the mean for the entire population, which was 1 ± 0.04 as compared with a mean of 7.5 ± 0.19 for a Pima population of 13 plants which had normally developed petal spots. Of a total of 106 flowers examined, in the 4-lock progenies, 16 on 14 different plants showed no trace of a petal spot, and none of the flowers was graded higher than 2.

Four progenies, descended from two of the individuals which were selected in 1917[1] for a high percentage of 4-lock bolls, were grown in 1920. Ten flowers on each plant in these four progenies were graded by the writer with respect to the degree of development of the petal spot, and the progeny means were computed from the mean grade of the 10 flowers per plant. The results are stated in Table IX.

TABLE IX.—*Mean grade of petal spot development in the 4-lock progenies of 1920*

Progeny.	Number of plants.	Number of flowers.	Mean grade of petal spot.
1–3–2	15	150	1.0±0.06
1–3–12	45	450	1.0± .04
3–2–4	38	380	.9± .04
3–2–5	42	420	.9± .03

[1] It is a remarkable coincidence that of two individuals which were selected as having a high percentage of 4-lock bolls, although at the time of selection this peculiar character of the flowers was not noted, the descendants have shown themselves to be alike in the subnormal development of the petal spot. As it is unlikely that this character would have been overlooked if it had been common to all plants of the strain in which these selections were made in 1917, the most probable explanation of the coincidence is that the two individuals in question were sisters.

Of the 1,400 flowers which were examined in these four progenies, 459, or 33 per cent, showed no trace of a petal spot, and 165, or 12 per cent, showed only the faintest discernible trace. Only 27 flowers were graded No. 3, and only 4 flowers were graded No. 4, the highest grade which was represented. None of the plants showed total absence of the spot in all 10 of the flowers which were examined, but on 1 plant each in progenies 1–3–2 and 3–2–4, 9 of the flowers showed no trace of the spot and the tenth flower showed only a faint trace (grade 1).

The range of variation in petal spot development shown by the great majority of the 4-lock plants in 1920 (from 0 to grade 2) as compared with the normal development in Pima, the total absence of the spot in the Holdon variety of Upland cotton, and a somewhat subnormal development in the first generation of a hybrid between Pima and Holdon, are illustrated in Plate 54.

The strongly hereditary nature of this variation is shown by the absence or slight development of the spot on all plants of the three progenies in 1919 and of the four progenies in 1920, whereas in both years all other stocks of the Pima variety which were growing in the same field and on which observation of this character was made showed a normal development of the petal spot.

In regard to other color characters of the flowers, the petals on every plant in the 4-lock progenies in 1919 and 1920 had the full lemon-yellow color and the anthers had the full orange-yellow color which is characteristic of Pima, and in all other characters, excepting the greater number of 4-lock bolls and the absence or very slight development of the petal spot, no appreciable departure from the type of the variety was observed.

The tendencies to increased lock number and to disappearance of the petal spot in these lines suggest that there may have been non-Egyptian "blood" in their remote ancestry. In most varieties of American Upland cotton, as well as in the Hindi or "Weed cotton" of Egypt,[1] 4-lock bolls predominate and the petal spot is absent. There had been no opportunity for hybridization with either of these types subsequent to the origin of the Pima variety, but the possibility is not excluded that the factors in question were introduced through the Yuma ancestors of the Pima parent, the Yuma variety having perhaps received them from its Mit Afifi progenitors.

This association of very weak development or total absence of the petal spot with a relatively high percentage of 4-lock bolls suggests the existence of a negative correlation between petal spot and mean lock number. Within the 4-lock populations, however, no significant correlation could be detected; and in the second generation of a hybrid between Egyptian and Upland cottons, of which 180 plants were grown at Sacaton in 1919, this pair of characters proved to be uncorrelated, the

[1] COOK, O. F. HINDI COTTON IN EGYPT. U. S. Dept. Agr. Bur. Plant Indus. Bul. 210, 58 p., 6 pl. 1911.

coefficient of correlation having been 0.01 ± 0.05. The means of 22 progenies of the third generation of this hybrid, grown in 1920, showed a positive rather than a negative but not a significant correlation, the coefficient having been 0.22 ± 0.14. The absence of a significant and negative correlation between these characters in the second and third generations of a known hybrid between Egyptian and Upland cottons would be evidence, if evidence were needed, that the association of a high percentage of 4-lock bolls with great reduction of the petal spot in these Pima lines is not attributable to recent hybridization with Upland cotton.

SUMMARY

Evidence is presented in this paper of the occurrence of heritable variations in the Pima variety of American Egyptian cotton, which is probably the most uniform variety of cotton now grown on an extensive scale.

Comparison of a progeny grown from seed of the parent individual of the variety with the present commercial stock proves that there has been significant improvement in the length and abundance of the fiber, as well as in the uniformity of these characters. This is shown by comparison of the means and coefficients of variation (Table I) and of the frequency distributions (Table II).

Indications of the occurrence of heritable variations have been obtained in roguing fields of the Pima variety, although the variations are much less numerous and are of much smaller magnitude than those which were observed in fields of the older Yuma variety.

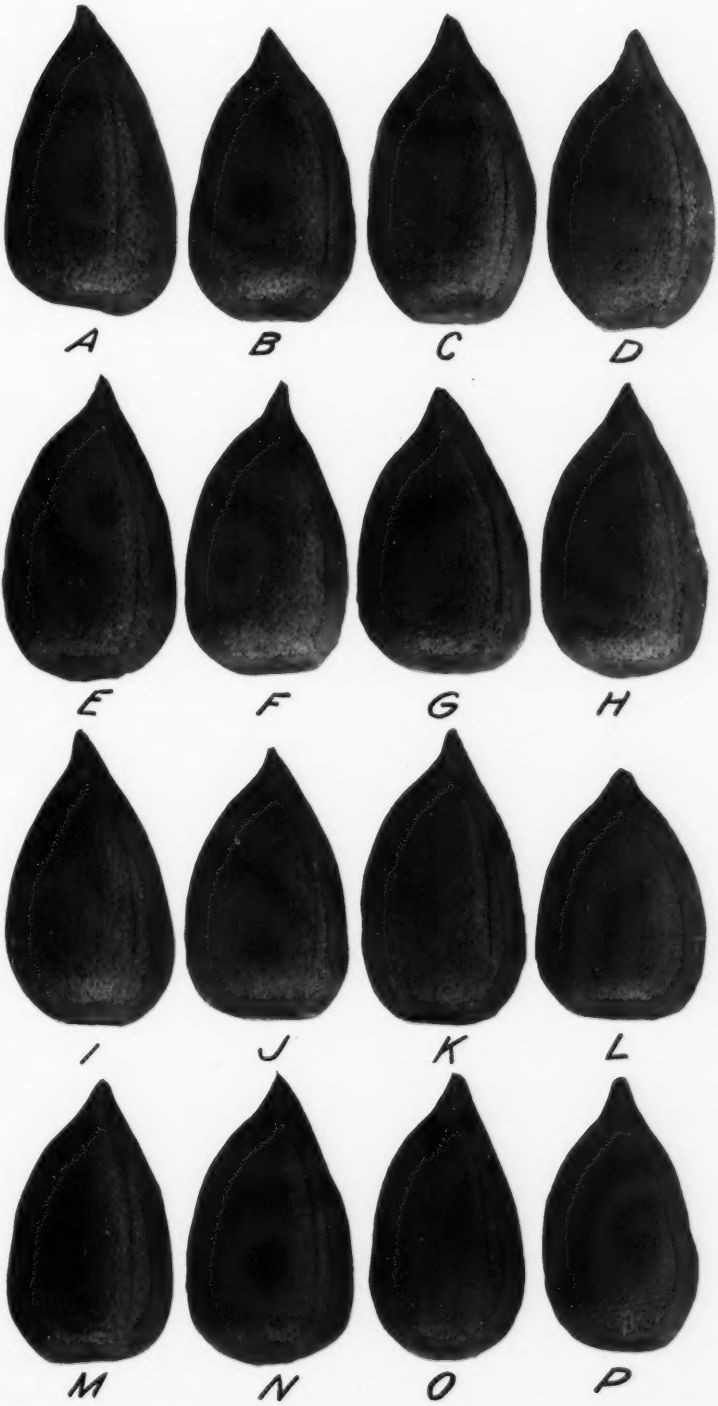
The records of the breeding work with Pima cotton supply additional evidence of the occurrence of slight, heritable variations, none of which could be considered as outside the normal range of variation of this variety. They indicate, however, that something may be accomplished by selection in regard to characters of practical importance.

A much more striking variation, characterized by the complete or nearly complete absence of the dark red spot near the base of the petal, associated with an increased percentage of 4-lock bolls, was found to be heritable in a high degree. The nature of this variation and the circumstances of its occurrence suggest the possibility that Upland cotton (*Gossypium hirsutum*) or Hindi cotton (*G. punctatum* Sch. and Thon.?) may have been involved in the remote ancestry of the Pima variety.

The fact that heritable variations are found in this apparently uniform variety is thought to justify the continuance of selection and line breeding and the roguing of seed increase fields.

PLATE 48

A progeny of 32 plants was grown in 1919 from seed of the original Pima plant of 1910, and an opportunity was thus afforded for determining the result of selection since the origin of the variety. Representative bolls, one from each plant of this "parent progeny" are shown in this and the following plate, plants 1 to 17 being shown in Plate 48 and plants 18 to 32 in Plate 49. All are within the normal range of the variety as it now exists, with the exception of Plate 48, L, and Plate 49, K. Slightly reduced.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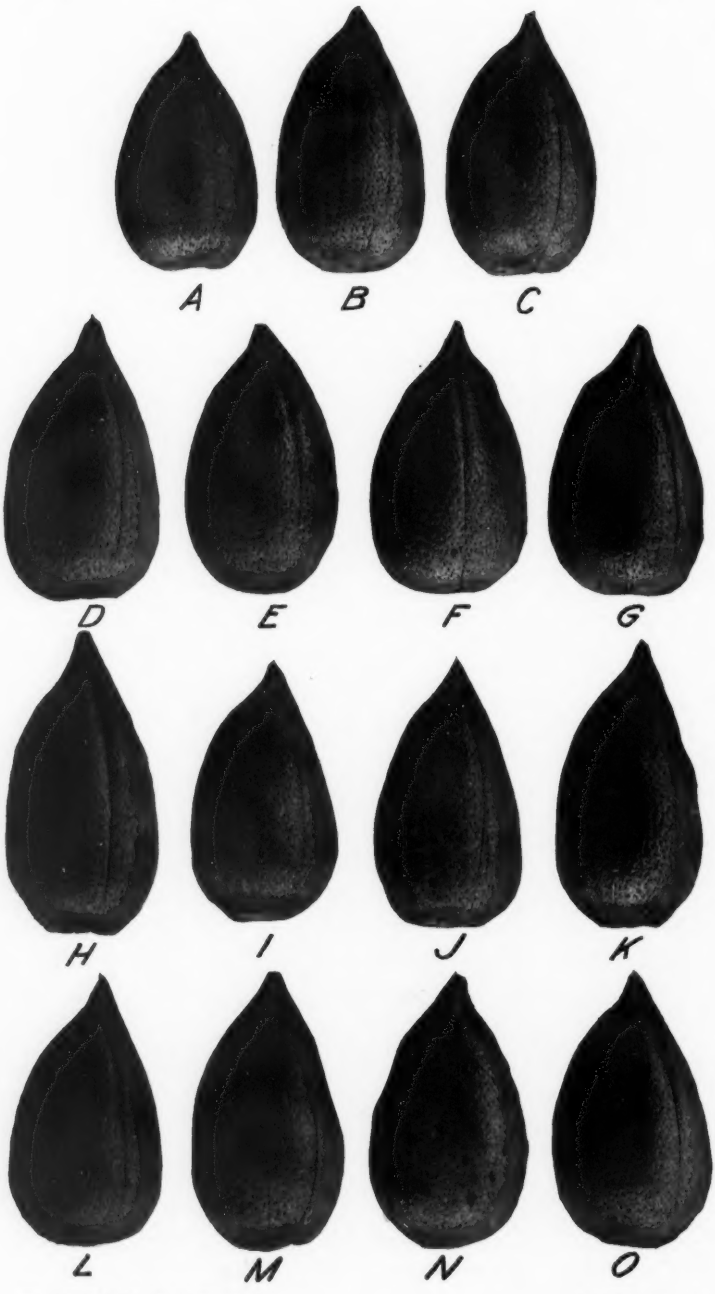
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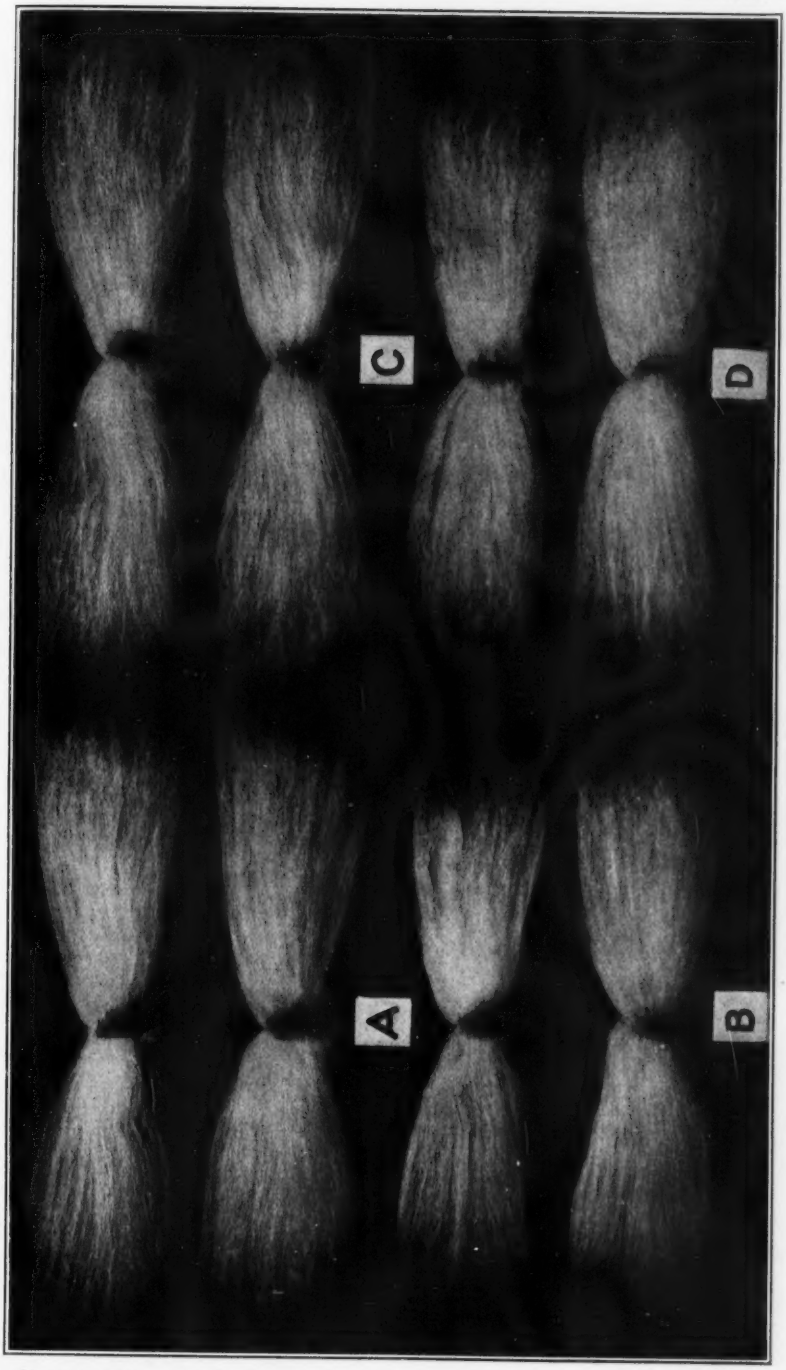
PLATE 49

Continuation of representative bolls, one from each plant of the "parent progeny" as shown in Plate 48.

PLATE 50

Seeds with combed-out fiber of Pima cotton grown in 1919, showing range in length of fiber: A and B from the plants in the progeny of the parent of the variety and C and D from the plants of the commercial stock (strain 5–3) which had the longest and the shortest fiber, respectively. The comparison shows a greater variation in length and a lower minimum length in the immediate progeny of the parent plant than in the present commercial stock.


C
D
A
B

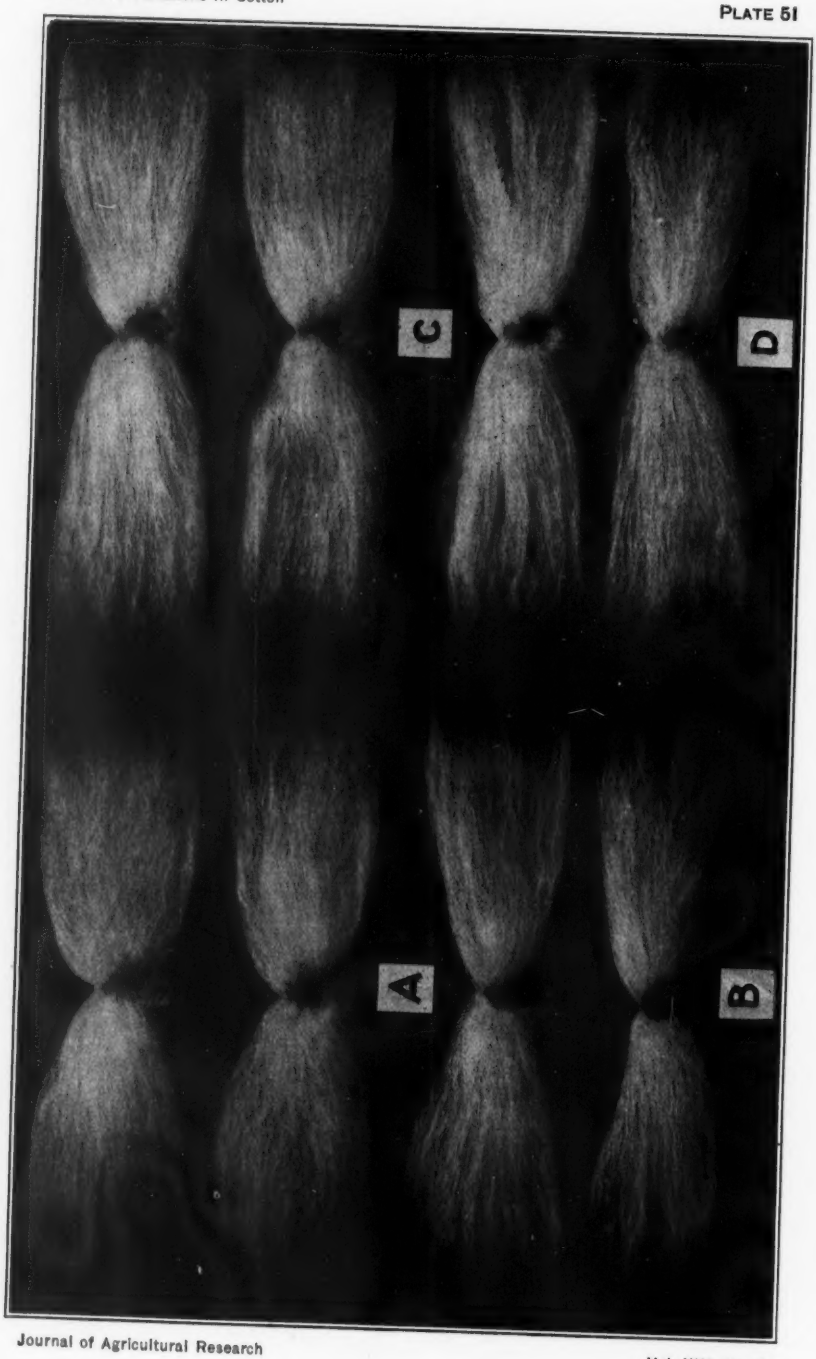

C
D
A
B

PLATE 51

Seeds with combed-out fiber of Pima cotton grown in 1919, showing range in abundance of fiber: A and B from the plants in the progeny of the parent of the variety and C and D from the plants of the commercial stock (strain 5–3) which had the highest and the lowest lint index, respectively. The comparison shows a greater variation in abundance and a lower minimum abundance in the parent progeny than in the present commercial stock.

PLATE 52

Seeds of Pima cotton grown in 1919, showing range in fuzziness: A and B from the plants in the progeny of the parent of the variety and C and D from the plants of the commercial stock (strain 5–3) which had the smoothest and the fuzziest seeds, respectively. The comparison shows a greater range in fuzziness and a nearer approach to naked seeds in the parent progeny than in the present commercial stock.

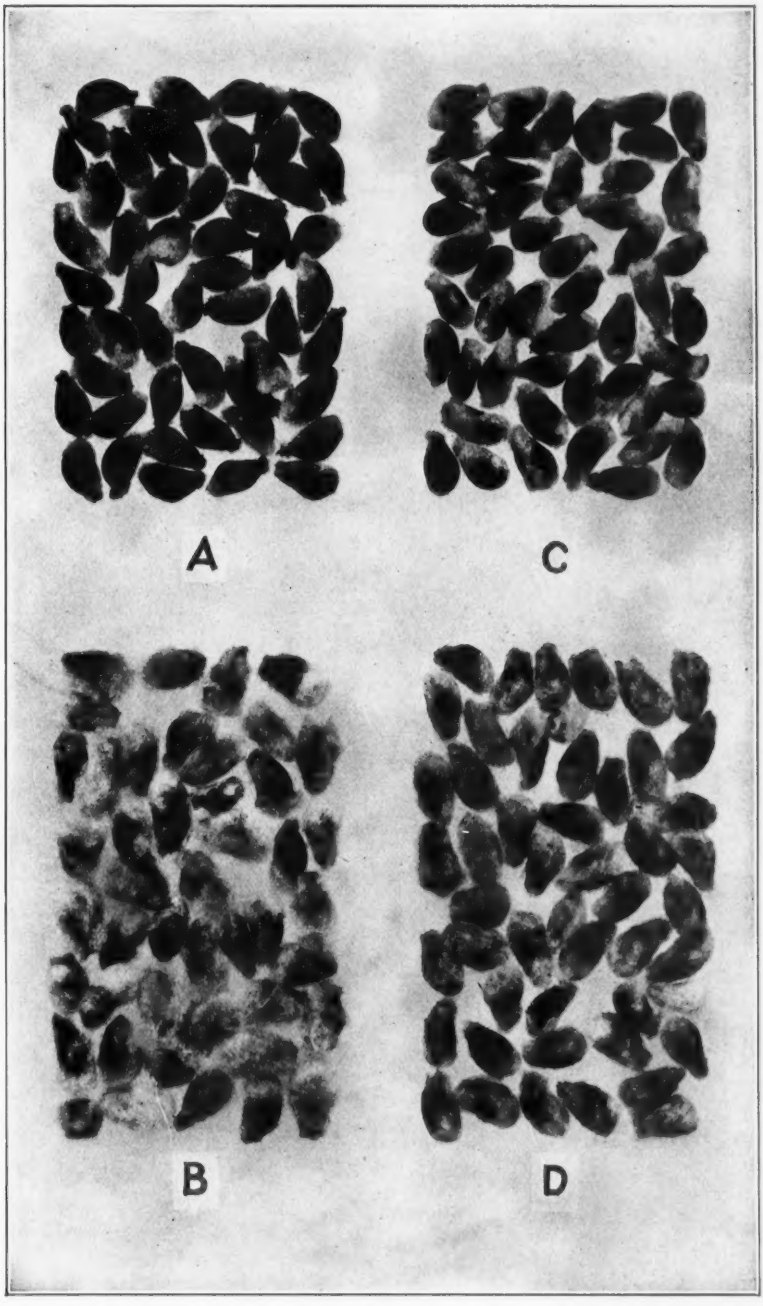
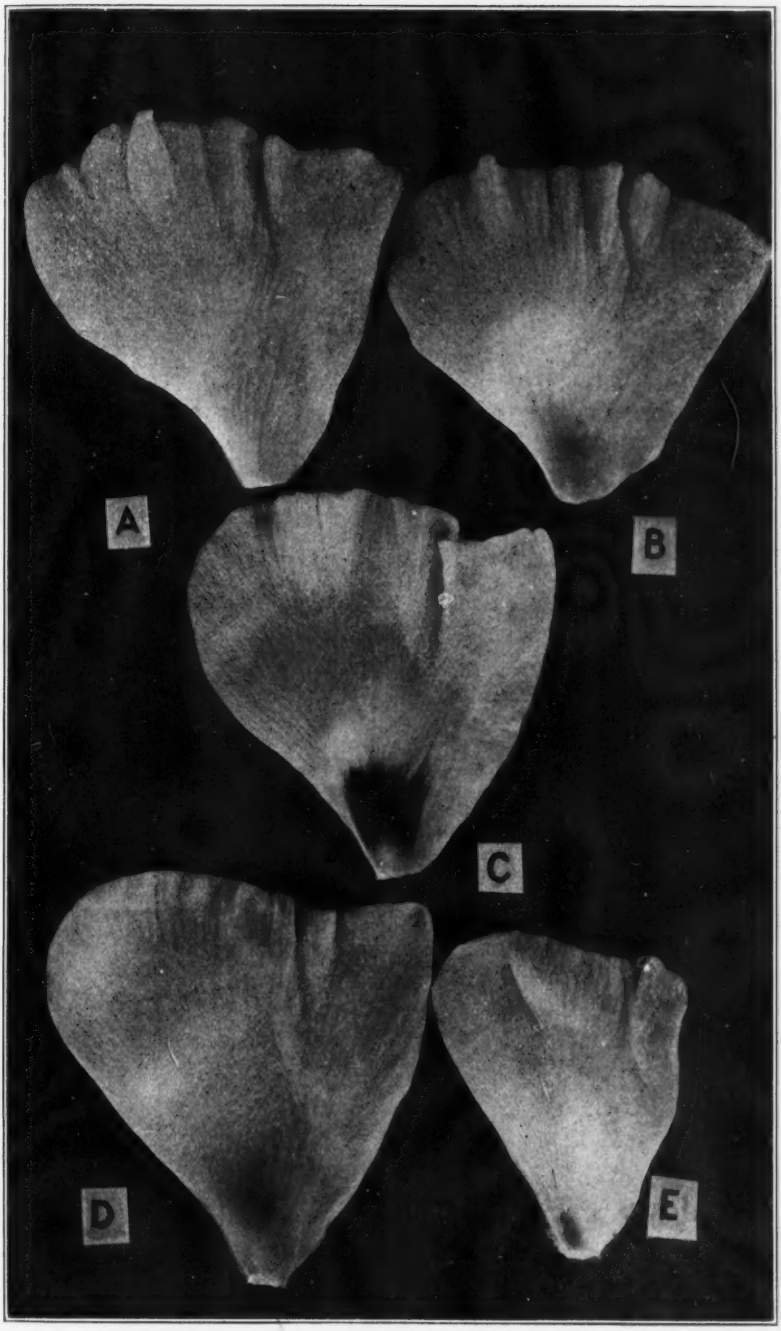

A
C
B
D

PLATE 53

A plant of the Pima variety of American Egyptian cotton, strain 5–3, photographed in August when the lowest bolls were nearing maturity. This plant represents the ideal which is sought in the breeding work with this variety, being free from vegetative branches or "limbs" and having fruiting branches well furnished with bolls at every node from near the base to the top of the stem.

PLATE 54

Comparison of petal-spot development in Pima 4-lock selections (A and B), normal Pima (C), the first generation of a hybrid between Pima Egyptian and the Holdon variety of Upland cotton (D), and Holdon (E). A and B represent the range for the great majority of the Pima 4-lock flowers, from total absence (grade 0) to about grade 2. C represents the full development in Pima (grade 8). D represents a rather weak development for an Egyptian-Upland first-generation hybrid (between grades 3 and 4). E represents the total absence of the spot in Holdon Upland.


A
B
C
D
E

SOME RELATIONS OF TEMPERATURE TO GROWTH AND INFECTION IN THE CITRUS SCAB FUNGUS CLADOSPORIUM CITRI[1]

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INTRODUCTION

In some previous work by the author (*3*)[2] it was found that the results of different inoculation tests on rapidly growing sour-orange trees (*Citrus aurantium* L. (*13*) with *Cladosporium citri* Massee were variable. It had previously been observed in the citrus orchards of Florida that abundant infections from scab did not inevitably follow the presence of abundant moisture on rapidly growing tissue, although these two conditions were usually present when abundant infection did occur. Seasons were encountered when scarcely any infection from scab occurred, even though conditions of moisture and growth appeared to be ideal for an outbreak. The author was led to suspect that temperature was also an important factor in infection.

The experiments which form the basis of this paper were planned to determine what influence different temperatures might have, first, upon infection by the fungus when the two other conditions previously mentioned, abundant moisture on the leaves and rapid growth were maintained, and second, on the growth and spore formation of the causal fungus on culture media. These experiments with the scab fungus carried on during intervals in a more extended temperature investigation with other citrus fungi reported elsewhere (*4*) are seen to be somewhat incomplete, but they appear to throw considerable light on the possible relation of temperature to the occurrence of citrus scab and to offer a more complete explanation for the differences in the occurrence of scab from year to year or from one season to another. It is, of course, realized that in the orchard, under natural conditions, temperature is fluctuating and not constantly maintained as in these experiments. Nevertheless, the experiments indicate at least the comparatively narrow range of temperature within which infection of a very susceptible host is possible under the presumably ideal conditions.

[1] Paper No. 72, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Reference is made by number (italic) to "Literature cited," p. 253.

Citrus scab has been described and illustrated in considerable detail in previous publications by Scribner (*8*, *9*), Swingle and Webber (*14*), Fawcett (*2*), and others. Its main characteristics are corky wart-like projections on leaves, fruit, and small twigs, due to the attack of the fungus on tender, rapidly growing tissue. The portion of the leaves not attacked usually maintains a healthy green color close up to the edge of these warts or scabs.

Considerable confusion has resulted, however, in the identity of the causal organism, *Cladosporium citri* (*1*, *2*). It should be mentioned that the fungus is an unusual Caldosporium and very different from the ordinary type of Cladosporium such as *Cladosporium herbarum* Lk., for example. This causal relation of *Cladosporium citri* to scab was at one time questioned by Grossenbacher (*5*), probably because he was working with another Cladosporium as indicated by the characteristics mentioned in his paper. The author's identification of *Clodosporium citri* as the causal organism has been confirmed by Hesler (*6*), Stevens (*12*), and Horne (*3*), as well as by his own later work (*3*). Moreover, *Cladosporium citri* has been found by Stevens (*10*) to be the cause of a similar disease known as avocado scab in Florida. Sour-orange (*Citrus aurantium*) (*13*) and lemon (*Citrus limonia* Osbeck) trees appear to be the most susceptible hosts to citrus scab. Satsuma orange (*Citrus nobilis* var. *unshiu* Swingle), trifoliata orange (*Poncirus trifoliata* Raf.), and pomelo (*Citrus grandis* Osbeck) are also attacked. Sweet orange (*Citrus sinensis* Osbeck) is nearly immune. In most of the infection experiments here reported, the sour orange was employed because it was one of the most susceptible species.

The experiments were made in 1917 at the Laboratory of Plant Physiology, Johns Hopkins University, in surroundings entirely free from the disease in question. The various temperatures employed were maintained for the most part by means of the apparatus described by Livingston and Fawcett (*7*).[1]

TEMPERATURES AT WHICH YOUNG CITRUS LEAVES WERE INFECTED

A preliminary test on sour-orange seedlings was made on May 16, 1917, with 8-months-old plants, which had been potted about three weeks and were beginning to develop two or three new leaves each. These plants were placed for three days in the different temperatures indicated below and were inoculated by drawing over the surface of the leaves and shoots a camel's-hair brush moistened with spores and bits of mycelium of the fungus in distilled water.

The average temperatures of the nine different chambers were 12°, 16°, 19°, 23°, 26.5°, 31°, 34.5°, 38.5°, and 42.5° C., with a fluctuation of 1° to 2°. One plant was exposed in each chamber for a period of three days in a saturated atmosphere and then taken out and the surface water

[1] The author wishes to acknowledge the aid rendered by Dr. B. E. Livingston in these experiments.

allowed to dry. The plants that had been inoculated at 16°, 19°, and 23° subsequently became diseased. The plant from the 16° chamber had scabs just visible 7 days from date of inoculation. Those from the 19° and 23° chambers were not seen to be diseased until 10 days later. The plants that had been inoculated at 16° and 19° had many scabs. The plant that had been inoculated at 23° was only slightly diseased even on the seventeenth day after inoculation.

Controls which were not inoculated, but on which distilled water was drawn over the leaves, were subjected to 16° and 26.5° C. and kept under the same conditions as the others, but no scab developed upon them.

A second test on sour-orange seedlings just like the first was made on June 5, 1917, except that different temperatures were maintained in some of the chambers and two plants were left in each temperature, and they remained for 54 hours instead of 3 days. Control plants not inoculated were used at 20° and 27.5° C. The average temperatures in this test were 14°, 20°, 24.5°, 27.5°, 32.5°, 36°, 40°, and 44.5°.

In this test one of the plants inoculated at 20° C. developed scabs on its leaves. These were first noted two weeks after inoculation. None of the other plants developed scabs. As will be seen in Table I, the chamber at 20° is the only one that lies in the range at which infection occurred in the other two tests, namely, 16° to 23°. The chambers at 14° and 24.5°, therefore, appeared to be outside, one below and the other above, the range for infection.

On September 24, 1917, a third inoculation test was made with sour-orange trees in pots, about one year from seed, on which small new leaves were developing. The plants were surrounded by cylinders of blotting paper kept moist by absorption of water from below. Another piece of blotting paper was placed over the top of the cylinder. The different temperature chambers (7) in which the plants were left for 3 days were maintained nearly constant within a fluctuation of about 0.5° C. At the end of this 3-day period when they were taken out, the moist cylinders of blotting paper were removed, and the plants were set in the greenhouse. In about ½ hour the moisture had evaporated from the surfaces of the plants. At the same time that these were taken out, two other plants were inoculated with the fungus as before and were set in the same greenhouse and under the same conditions as the others. These were not surrounded by blotting paper but were allowed to dry like the others, after water with spores had been drawn over their leaves.

The temperatures at which the plants remained during the 3-day period in the saturated atmosphere of the dark chambers were 13.5°, 16°, 18.5°, 21°, 24.5°, 27.5°, and 32° C., respectively.

The temperatures recorded in the greenhouse for the 12 days after the plants were taken out of the temperature chambers gave an average minimum of 15° C., and an average maximum of 30°.

Only those subjected to 16°, 18.5°, and 21° C. for the 3 days after inoculation developed any scab on their leaves after removal to the greenhouse benches.

On plants inoculated at 16° C. scab was just visible in 6 days from the time of inoculation, or 3 days from the time of removal from the temperature chamber. In 2 weeks the scab lesions were well marked.

Plants inoculated at 18.5° C. showed a slight indication of scab 5 days after inoculation, or 2 days after plants were removed from the temperature chamber. In this case the scab lesions were well marked in 11 days from time of inoculation.

Plants inoculated at 21° C. showed a slight indication of scab 5 days after inoculation, as was true of those at 18.5°. In 7 days the diseased spots were quite distinct, and in 11 days they had developed into definite and typical scabs. On plants inoculated at 13.5°, 24.5°, 27.5°, and 32°, however, no sign of lesions developed.

Control plants were also kept under the same conditions, but none of these developed scab lesions at any time. The two plants which were inoculated with the fungus at the time the others were taken out of the temperature chambers and from whose surfaces the water was allowed at once to evaporate also remained free from scab. These were considered to be additional controls. The nonappearance of scab on these two plants indicated that infection at exposures of 16°, 18.5°, and 21° C. developed because the hyphae had penetrated while the plants were still in the temperature chambers. This conclusion is also supported by the fact that the time from inoculation to the appearance of visible scabs agrees well with that observed in previously reported tests (*2*, *3*). The time elapsing between the removal of the plants to the greenhouse bench and the first appearance of scab appears generally to be too short to represent the incubation period of the fungus within the tissues of the host.

On September 28, 1917, a set of young pomelo seedlings with leaves just begining to unfold was inoculated in the same manner as that described for the sour-orange seedlings of the third test, except that these were left four days in the temperature chambers instead of three. With the exception of one leaf on a plant inoculated at 18.5° C., which developed a slight indication of disease but no distinct scab, there was no visible sign of disease on any of the plants up to October 17, when the last observation was recorded.

In all four tests just considered, scabs were always confined to rapidly growing leaves. The older leaves always remained free from the disease in every test. Neither was there any development of scabs on any of the large number of similar plants kept in the greenhouse and not inoculated.

TABLE I.—*Range of infection as indicated by the four inoculation tests on citrus seedlings*[1]

Plant and date of inoculation.	Results of inoculation at temperature (°C.) of—																						
	12	13.5	14	16	18.5	19	20	21	23	24.5	26.5	27.5	31	32	32.5	34.5	36	38.5	40	42.5	44.5
Sour orange:																					
(1) May 16, 1917	o	....	...	*	....	*	....	....	*	....	o	....	o	....	....	o	...	o	...	o	....
(2) June 5, 1917	....	....	o	...	....	....	*	....	...	o	....	o	....	....	o	....	o	....	o	....	o
(3) Sept. 24, 1917	....	o	...	*	*	....	....	*	...	o	....	o	...	o	....	....	....	....	....	....	....
Pomelo:																					
(4) Sept. 28, 1917	....	o	...	o	*	....	....	o	...	o	....	o	...	o	....	....	....	....	....	....	....

[1] * Indicates definite infection; o, no infection.

INOCULATION OF DETACHED LEAVES

Young sour-orange leaves were detached and placed in Petri dishes, one set containing distilled water on which the leaves were floated and the other set containing cornmeal agar. Some of the fungus mycelium was placed in contact with the leaves, and the preparations were allowed to remain in the temperature chambers for 15 days. The temperatures used were 13.5°, 16°, 18.5°, 21°, 24.5°, 27.5°, and 32° C., with a fluctuation of about 0.5°. Infection took place in water at 16°, 18.5°, 21°, 24.5°, and 27.5°, but not at 13.5° nor at 32°; and in cornmeal agar at 18.5° and 21°, but not at 13.5°, 16°, 24.5°, 27.5°, nor 32°.

INFLUENCE OF TEMPERATURE ON GROWTH AND SPORE PRODUCTION OF CULTURES

In addition to the inoculation experiments described above, the rate of growth and the formation of spores were studied with different maintained temperatures. Distilled water, in which was floating a young sour-orange leaf, and cornmeal agar were used as culture media. Inoculation was accomplished by means of a disk (2.5 mm. in diameter) of agar medium bearing the mycelial weft. The cultures were kept in the dark, maintained temperature chambers for three or four days. At the end of the period the radial extension of the hyphae from the transferred disk, was determined. Observations were also made on the general abundance of spores. The data obtained are brought together in Table II. The growth rate is seen to have been very slow as compared with that of many other citus fungi (*4*). For the time employed and for the temperatures used the greatest extension of hyphae occurred at 21° C. This growth rate was smaller, for each temperature, in cornmeal agar than in distilled water with the leaf, except at the two higher temperatures. At 32° no extension of hyphae was seen in water, while in cornmeal agar the enlargement was about one-sixth of that obtained at 21° for the same period.

Spores were observed on the marginal hyphae in from 24 to 48 hours in all cases where growth was observed, except in the agar culture at 32° C. The first examination for presence of spores was made after 48 hours in the agar cultures. Spores were abundant at the first examination upon the growing hyphae of all the test cultures at 21°, one of the temperatures

at which infection of rapidly growing sour-orange seedlings and detached sour-orange leaves had been observed to be pronounced. Spores were also abundant in the water preparations at 24.5° and 27.5° after 24 hours, and in the preparations of agar at 24.5° after 48 hours; at these temperatures no infection had occurred in the tests with growing plants, but a slight infection had been observed after 10 days on detached leaves in water.

Spores that had fallen from the hyphae in certain ones of these cultures were found to have fallen on the surface of the medium at some distance laterally from the ends of the aerial hyphae on which they had been borne. Apparently these spores had been ejected with considerable force from the ends of the hyphae. In one case at 16° C. a fringe of spores was observed, most of them at least 210 microns distant from the ends of the outermost hyphae. This feature of spore dispersal in cultures had previously been seen but not recorded. It was noted as most frequent after four days in cultures growing at the edges of leaves in cornmeal agar at 16°, 18°, 21.5°, and 24.5°.

In the 16° preparation with water and a sour-orange leaf, spores were observed to be mostly formed on the tip ends of the outwardly extending aerial hyphae at some distance from the floating leaf. They were nearly hyaline at first, subsequently becoming slightly dusky. Most of them were 1-celled, but a few were 2-celled. Detached spores were germinating from the ends of spores in line with the longest axis. Hyphae were hyaline when viewed singly, but were pinkish or flesh-colored in mass. On cornmeal agar under the same conditions spores were forming with from two to four in a chain, and the ejected spores were germinating on the surface of the medium.

The influence of temperature on the vegetative hyphae was marked. At 27.5° and 32° C. in the cornmeal agar preparations the hyphae were broad and straight, 8 to 12 microns or more in diameter. At 23° and 21°, on the other hand, the hyphal diameter was only one-half as great and the hyphae were more bent and tortuous. At 18.5°, 16°, and 13.5°, the hyphae were broader, much as at the higher temperatures.

TABLE II.—*Growth and spore formation in Petri-dish cultures of Cladosporium citri at different maintained temperatures*

Temperature.	Fungus floating in water with tender leaf.			In cornmeal agar.		
	Radial growth in 3 days.	Spore formation.		Radial growth in 4 days.	Spore formation.	
		In 1 day.	In 3 days.		In 2 days.	In 4 days.
°C.	*Microns.*			*Microns.*		
13.5	62	Few.....	Few.....	38	Few.....	Few.
16	170	...do.....	Abundant..	92	...do.....	Do.
18.5	285	...do.....	Few.....	116	...do.....	Do.
21	655	Abundant..	Abundant[1].	370	Abundant[1].	Abundant.
24.5	385	...do.....	Abundant..	323	...do.....	Do.
27.5	154	...do.....	Abundant[1].	197	Few.....	Few.
32	0	None.....	None.....	62	None.....	None.

[1] Spores found some distance laterally from ends of the outermost hyphae as if ejected with considerable force.

TABLE III.—*Number of days from inoculation to first indication of disease at different temperatures* [1]

	Days after inoculation at temperature (°C.) of—								
	16	18.5	19	20	21	23	24.5	26.5	27.5
Sour-orange seedlings:									
(1) May 16, 1917	7	—	17	—	—	17	—	—	—
(2) June 5, 1917	—	—	—	14	—	—	—	—	—
(3) Sept. 24, 1917	6	5	—	—	5	—	—	—	—
Detached leaves:									
(4) In cornmeal agar	—	5	—	—	4	—	—	—	—
(5) In water	6	4	—	—	3	—	10	—	10

[1] A dash indicates that no scabs developed on plant placed at the respective temperatures. A blank space indicates that no plants were used in a given test at these respective temperatures.

DISCUSSION

Infection of sour-orange seedlings was limited to a range of about 8° C., 16° to 23°, inclusive, in these experiments. This range could not have been over 10° in extent under the same conditions even if more temperatures had been investigated, since plants inoculated at 14° and at 24.5° failed to become diseased. This range is seen to include the temperature (21°) at which the fungus was found to have the most rapid growth in water and in cornmeal agar medium. The temperatures just outside the range on the lower side (14° and below) are those at which the fungus grew somewhat more slowly than it did at the temperatures just outside the range on the upper side (24.5° and above). If, however, we consider the experiment with detached leaves in water (Table III) in which an exposure of 15 days was given at the different temperatures, the range for infection, though not extended downward, was extended upward to 27.5°, a temperature at which the hyphal growth in water was approximately the same as that at the lower limit of the range, 16° (Table II). This may indicate that the rate of extension of the hyphae must be above a certain minimum before infection of the host can occur, a point that may have some significance. The rate of extension of the hyphae also appears to have a relation to the time between inoculation and first appearance of scab as is shown, in a general way, in Table III. This time period, with a single exception, is shortest with temperature at or near 21°, the temperature for maximum growth if the tests on different dates be considered separately.

It is, of course, not to be definitely concluded that infection can not occur with temperatures outside the range within which it was confined in these experiments, but it appears probable that scab is not to be expected with temperatures that remain outside this range. Of course, orchard temperatures may vary so as to subject the trees to temperatures within the range of infection for a sufficient time period to allow the penetration of the fungus. No attempt was made to determine

the minimum time necessary for the fungus to become established in the host tissue at the different temperatures listed in order to produce scab subsequently.

The interrelation of the host and parasite also demands consideration. Temperature, of course, influences the host as well as the parasite, and the lack of infection in these experiments at certain temperatures may have been due to special physiological conditions or states either in the host or in the parasite, or even in both. It may be suggested that there was not enough time for infection in those cases where it failed to occur. From the experiments in which leaves floating in water were left for a long time in contact with spores of the fungus at different maintained temperatures, it appears that, even with long periods of time, there are certain temperatures, not fatal to either parasite or host, at which infection does not take place. At some temperatures the fungus even grew fairly well on the surface of the leaf without producing infections leading to scab.

The relation of temperature to infection by the scab fungus, as here brought out, seems to suggest a satisfactory explanation for some differences in the results of inoculations previously recorded by the author (*2*). A set of inoculations made in August, 1906, failed to produce scab, while a similar set made in January, 1909, was successful. An examination of the weather records for the locality where the experiments were made shows that the mean daily temperatures for the two weeks following the August experiment fluctuated between a minimum of 23° and a maximum of 30° C., while the corresponding temperature range for the January experiment lay between a minimum of 11° and a maximum of 19°. In the former case, the daily means were above the range for infection of seedlings in the experiments here reported except at its minimum, 23°, while the other case shows an overlapping of the range of daily means with the range for infection, to 19°. Since the temperatures in the greenhouse where the 1909 experiments were performed were probably higher than those outside, the successful infections in January were almost certainly due to the fact that the temperatures fell for the most part entirely within the range that is favorable to infection.

The temperature relation here emphasized is probably important in determining the occurrence of scab in the orchard and in the explanation of scab epidemics. But temperature is only one of a number of conditions, such as stage of growth, humidity, time period, etc., that must be fulfilled for scab to occur.

Among the various conditions discussed by Grossenbacher (*5*) as favoring the development of scab, the following general observations seem to bear on this temperature relation.

If the air is fairly dry and the weather mostly warm and bright during the development of the first spring flush, scab may fail to develop. On the other hand, the disease

often becomes very severe in groves if the weather is cold and wet during the development of the first spring growth.

Again:

Some trees in scabby groves under observation during the past two years have retained the late starting habit and remained practically free from the disease.

Again:

During the early spring of 1915 the air was very moist and cold while the first growth was in its early stages and as a result sour scab developed in great abundance even on high sandy land if growth was early and vigorous.

Stevens (*10*) says of citrus scab:

If cool wet weather prevails at the time the new growth is putting out or at the time the fruit has set, the disease is apt to be severe . . . in groves where scab has become established.

In the light of these temperature experiments, the greater severity of scab at the low spring temperatures of Florida seems to be due largely to the fact that these temperatures fall mainly within the range for infection, while the later spring and summer temperatures are usually too high for infection to take place, even though other conditions are favorable. An examination of the temperature data for Tampa, Fla., given in Table IV suggests that this last statement may be translated into terms of mean temperatures, to the effect that severe infection will usually occur when the mean temperatures are well within the infection range and that infection will be unlikely to take place when the mean temperatures are outside this range.

TABLE IV.—*Mean temperatures at Tampa, Fla.*

[In degrees centigrade]

Month.	1909	1910	1911	1912	1913	1914	1915	1916	1917	1918
February	16.5	15.5	17.5	13.5	18.5	15.5	15.5	16.5	16.5	20
March	19.5	19	20	20.5	21	16.5	14.5	17	20.5	22
April	23	20.5	23	23	21	22	20.5	20.5	22	22
Mean	19.5	18.5	20	19	20	18	17	18	20	21
May	24.5	24.5	24.5	25.5	24	25	26	25	24	24.5
June	27	26.5	27	26	26	27	28	26	26.5	27
July	26.5	26.5	27	28	27	27	28.5	28	28	28
Mean	26	26	26	26.5	25.5	26.5	27.5	26.5	26	26.5

The average of the monthly mean temperatures for February, March, and April for this 10-year period was between 17° and 21° C., while the average of the monthly mean temperatures for May, June, and July was between 25.5° and 26.5°. All the monthly mean temperatures for the first period were well within the infection range for scab, while all the

monthly means for the second period are well above the infection range as determined by the inoculation experiments.

It is of interest to note also that the lowest average for February, March, and April was 17° C. in 1915, the year mentioned by Grossenbacher (*5*) as one in which scab developed in great abundance on the growth starting in early spring. These data taken in connection with the maintained temperature experiments seem to explain the frequent absence of scab on tender growth of the second or third cycles even in the moist periods of summer. The spores may be present, the moisture and growth conditions may be favorable, but the temperature may be too high.

The occurrence of severe late scab in some seasons is probably due to a decided drop in the temperature, bringing it well within the infection range for a sufficient length of time for infection.

SUMMARY

(1) Experiments to determine the influence of temperature on scab infection on young sour-orange (*Citrus aurantium*) leaves and on the growth and spore formation of the causal organism (*Cladosporium citri*) are reported.

(2) The inoculation temperatures resulting in infections of growing plants under conditions of rapid growth and abundant moisture were 16°, 18.5°, 19°, 20°, 21°, and 23° C. No infections were obtained under the same conditions on plants inoculated at 12°, 13.5°, 14°, 24.5°, 26.5°, 27.5°, 31°, 32°, 32.5°, 34.5°, 36°, 38.5°, 40°, 42.5°, and 44.5°.

(3) Detached leaves floated in water with the scab fungus were infected at 16°, 18.5°, 21°, 24.5°, and 27.5° C.

(4) The temperature at which the greatest extension of hyphae of the causal organism in cultures was observed was 21° C. The highest temperature at which extension was observed in water was 27.5°, and in cornmeal agar 32°.

(5) Spores were observed in 48 hours or sooner in all the temperatures at which growth took place except 32° C. At certain temperatures the spores appeared to be ejected with considerable force from the ends of the hyphae.

(6) The temperature at which the time was shortest between inoculation and first observance of signs of disease was usually 21° C. in the different tests. This time increased toward the upper and lower limits of the infection range.

(7) This limited range of temperature at which infection of a susceptible host took place under the presumably favorable conditions of the experiment appears to explain the great differences observed in the occurrence and severity of scab from year to year and from season to season in citrus orchards. It also explains the differences in results of previous inoculation experiments not hitherto understood.

(8) The conditions necessary for scab infection indicated by these experiments are (1) viable spores of the fungus, (2) young citrus leaves of a susceptible species, (3) moisture, and (4) temperatures between 16° and 23° C.

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BACTERIAL WILT OF CASTOR BEAN (RICINUS COMMUNIS L.)

By ERWIN F. SMITH, *Pathologist in Charge, Laboratory of Plant Pathology,* and G. H. GODFREY, *Pathologist, Office of Cotion, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*[1]

HISTORY AND DISTRIBUTION

The bacterial wilt of castor beans was first brought to the attention of the writers in May, 1918, when specimens of diseased plants were received from Townsend, Ga., where they were collected by Mr. I. C. Jagger. Isolations made from this material were used immediately for cultural studies and for numerous inoculations as already reported.[2] Soon after the first observation of the disease in the field, by direction of the Office of Cotton, Truck, and Forage Crop Disease Investigations, a survey was undertaken by Mr. Jagger of castor bean plantings in the South, particularly in Florida. The survey extended over a period of about two weeks and covered practically all sections of the State of Florida. In addition, reports of the occurrence of the disease were received from other men in Florida and other States.

The disease occurred at or near the following points in Florida: Monticello, Jacksonville, St. Leo, Ocala, Orlando, Plant City, Tampa, Lucerne Park, Winter Haven, Mulberry, Fort Meade, and Frostproof; at Townsend, Ga.; and at Dothan, Ala. The loss varied considerably in these localities, very exceptionally exceeding 10 per cent and varying from that down to zero. The highest reported loss was 30 per cent in a field in southern Georgia.[3]

On a trip made by the junior author early in August, freshly wilted plants were still to be found in the fields. During this survey the wilt was observed definitely at Orlando, Tampa, Seffner, and Fort Meade, Fla., and at Dothan, Ala. It is very likely that the disease occurred also in North and South Carolina and in Mississippi. The descriptions given by farmers of an early disease on the plants indicated this, but no authentic material was received.

A thorough search by both Mr. Jagger and the junior writer at different times among the heavy plantings along the east coast of Florida, from Miami to New Smyrna, failed to show the disease to be present. These observations were made at Miami, Little River, Arch Creek, Davie, West Palm Beach, Vero, Deer Park, Melbourne, Titusville, Mims, and New Smyrna.

[1] The field observations on this disease were wholly in the hands of Mr. Godfrey. The identification of the organism was made by the senior writer, and the inoculations were under his direction.

[2] SMITH, Erwin F., and GODFREY, G. H. BROWN ROT OF SOLANACEAE ON RICINUS. *In* Science, n. s. v. 48, no. 1228, p. 42. 1918.

[3] Reported by Mr. T. B. Young, of the Office of Drug Plant Investigations.

Journal of Agricultural Research,
Washington, D. C.
XW

Vol. XXI, No. 4
May 16, 1921
Key No. G-233

The east coast section differs from the interior and west coast sections of Florida rather strikingly in that an abundance of lime is present in the soil, cropping out here and there. Therefore this type of soil is probably in the main alkaline, as opposed to the more or less acid soil of the pine flats and the more rolling pine lands of the interior. It is suggested, therefore, that further studies of the distribution of *Bacterium solanacearum* EFS be made in Florida with special types of soil in mind to see if there is anything in this suggestion.

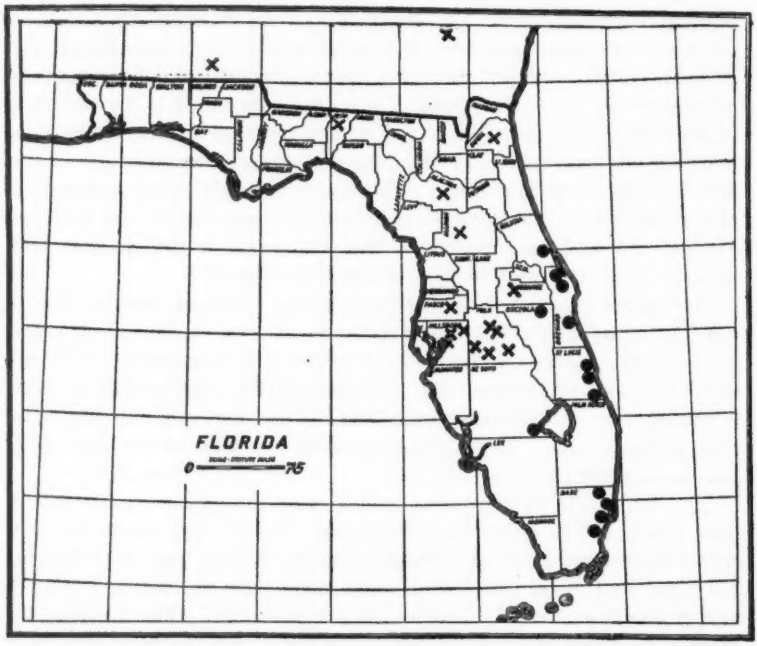


FIG. 1.—Map showing distribution in Florida in 1918 of Ricinus wilt due to *Bacterium solanacearum*. X indicates localities where the disease was found; ●, localities where the disease could not be found.

Late in the season plantings in the Everglades muck land, in the vicinity of Moorehaven, were inspected without any signs of the wilt being found. The outline map (fig. 1) shows the distribution of the wilt in Florida, as well as the points where the disease was searched for and not found, showing the sharp division of the State into two distinct regions.

Another observation on the soil type and its apparent relationship to wilt, as reported by Mr. Jagger, is to the effect that the newer land was more likely to show the wilt than land that had been longer under cultivation. This was observed on numerous occasions in the sections where wilt occurred. One or two particular cases may be mentioned.

On one field of 295 acres at Ocala, Fla., this contrast was particularly striking. No wilt whatever was observed in the main part of the field, which had been under cultivation for many years. On a new section of the field, which had been cultivated only two years and which adjoined pine woods, wilt occurred in varying amounts, running from one-tenth of 1 per cent to 10 per cent in different spots. It was typical rolling pineland with little humus. The older land was originally the same but had developed considerably more humus and a darker color.

A field at Frostproof, Fla., was inspected on June 6. This field was made up entirely of new land cleared and plowed in January and February, 1918, and planted in March. The wilt occurred in certain areas, especially in the lower portions of the field, to the extent of 5 to 10 per cent. Several other fields in the same general locality, which had been under cultivation for several years, showed a complete absence of the wilt.

At Mulberry, Fla., a plot of 80 acres of low pineland, with saw palmetto in the lower spots, was cleared in the latter part of February and planted March 13. While this gave an excellent stand of castor beans, wilt occurred throughout to the extent of one-tenth of 1 per cent to 5 per cent. A few square rods planted early in May (very late) showed numerous wilted plants.

The junior writer likewise inspected a field in Dothan, Ala., in which the only wilt to be found was in a newly cultivated end of the field adjoining pine woods.

The senior author has frequently observed this disease in tomatoes grown on "new land" in Florida.

Due to the fact that there were no extensive commercial plantings in 1919, the only observations on the disease in 1919 were in some experimental plantings made by the junior author at Orlando, Fla. As early as April the first cases of wilt were to be found, and from that time on till at least June 16 new cases were appearing almost daily. These cases all occurred on sandy land which had not been under cultivation for some years.

SIGNS OF THE DISEASE IN THE FIELD

The signs of the bacterial wilt on Ricinus are, in general, the same as on other plants described in detail by Dr. Erwin F. Smith, in "Bacteria in Relation to Plant Diseases,"[1] and in earlier publications. The disease in this plant, as in the Solanaceous plants, is a true wilt of the green leaves and growing points, without previous yellowing or other partial or complete discoloration (Pl. 55). It may occur when the plants are small, only 2 or 3 inches high, in which case it is likely to prove fatal. It is also found in plants of all sizes up to several feet high. In smaller plants a single leaf may wilt, and the vascular system near the base of the plant

[1] SMITH, Erwin F. BACTERIA IN RELATION TO PLANT DISEASES. v. 3. Washington, D. C. 1914.

on that side may show internal browning, or again the entire plant may droop. The wilting may come about very suddenly or slowly. The first manifestation of wilt often appears after a period of wet weather of several days' duration, such weather apparently favoring infection. More of the wilt is seen in hot, dry weather. Often a plant may recover temporarily during moist weather or during a cool night after a hot, dry day, which has resulted in the appearance of wilt. Sometimes this recovery leaves a shriveled tip or margin on some of the leaves. The ultimate result of the wilt, in the more severe cases at least, is the complete death of the plant. After wilting the leaves dry and turn black, later dropping from the plant and leaving the bare black stalk and branches.

In many plants infection was present late in the season but was not severe enough to cause permanent wilting or death of the plant. In such cases marked dwarfing occurred. In a field at Dothan, Ala., it was easily possible to pick out infected plants that showed no external signs other than dwarfing. Plate 55, A, reproduced from a photograph taken at Dothan, Ala., on August 24, 1918, shows from left to right, a dwarfed and a wilted plant (both of which showed unmistakable internal signs of the bacterial wilt) and a large normal plant.

A disease due to a root trouble and entirely distinct from the wilt, but showing some signs similar to it, was found in the vicinity of Miami, Fla., and at other points especially in muck lands. These plants appeared to be affected by asphyxiation of the roots due to too high a water table. Many such plants were observed with the root systems entirely dead and often with the tap roots rotted off 3 or 4 inches below the surface of the ground. Such root conditions resulted in the death of the top of the plant, manifested by wilting and drooping of the leaves and twigs and finally by the death and blackening of the entire plant (Pl. 56).

The bacterial wilt can be distinguished easily from the disease described above and from any other known disease of the castor bean by the typical browning of the vascular system near the base of the plant. In the severer cases this browning is seen in the entire vascular ring, penetrating more or less deeply into the woody part of the stem. In cases of light infection, such as in the dwarfed plants mentioned heretofore, the browning may be seen in only a few bundles, all possibly on one side of the stem. In fact, cases were observed in which only a single bundle gave this manifestation of the presence of disease. In addition to the browning it is always possible with the aid of a good hand lens to observe drops of bacterial exudate at the freshly cut ends of the vascular tubes, particularly if the plant is still green. Sometimes it is necessary to pinch the end of the stem to force these drops to the surface.

ISOLATION OF THE CAUSAL ORGANISM

Such materials as have just been described will almost invariably produce pure cultures of the causal organism when proper precautionary measures against external contamination are taken. Repeated cultures were made by flaming momentarily the green stalk from a wilted plant, then cutting it off with a sterile scalpel and squeezing out by means of strong forceps a drop of the plant juice together with the bacteria into the tube of beef agar. From this, dilutions were made and then plates were poured. This was varied by sterilizing the surface of a stalk with mercuric chlorid 1 to 1,000, girdling it with a circular cut and breaking it off, after which sterile conditions were still further insured by searing the edge of the freshly made cut with a red-hot scalpel. A hollow could now be made safely in the end of the stalk and a few loops of beef broth inserted and mixed with the plant juices. From this very thick suspension of bacteria, dilutions were then made for the poured plates.

RESULT OF INOCULATIONS

As soon as results from the poured plates gave indications that the organism in the diseased Ricinus plants was *Bacterium solanacearum*, inoculations were made into tomato plants, which wilted promptly. Cultures in other media such as nitrate bouillon (where reduction took place) and pink litmus milk (which became and remained blue) also served to confirm the diagnosis. From the interior of one of the wilting tomato plants Petri dish agar poured plates were made and subcultures from some of the colonies were tested out on other tomatoes which also wilted (Pl. 57).

By this time Ricinus plants large enough to inoculate were available, and a series of inoculations were made by needle on the hypocotyls. On these plants the progress of the disease was slower than on the tomato, but there was profound dwarfing as a result of the inoculations, and subsequently the plants wilted with bacterial occupation and browning of parts of the vascular system (Pl. 58–60). From such plants also the organism was plated out in practically pure culture. Large Ricinus plants also were successfully inoculated by needle pricks (Pl. 61). Subsequently the disease was produced on Ricinus by breaking some of the roots in soil infected by burying in it Ricinus plants already inoculated and swarming with the bacteria (Pl. 62).

We also obtained successful inoculations on a series of jimson weeds (*Datura stramonium* L.), the phenomena in which corresponded exactly to results formerly obtained by the senior writer with *Bacterium solanacearum* plated from other plants—that is, wilting, vascular infection, brown stain, etc.

The Ricinus organism was also inoculated successfully into the common nasturtium (*Tropaeolum majus* L.), another plant subject to *Bacterium solanacearum*.[1]

Subsequently it occurred to the senior writer to try out the organism on cotton, vanilla, and sunflower—three plants hitherto untried.

Cotton plants when of any size proved resistant, but the young seedlings are subject to the disease. Thus, if inoculations are made by needle pricks into the hypocotyl soon after the plants appear above ground they are first profoundly dwarfed (Pl. 63) and then wilted and shriveled (Pl. 64). Bacteria were then found in enormous numbers, at least in the parts above ground, the needle pricks being made near the cotyledons. These results were obtained in 1918, and the same results were obtained again in 1920, using *Bacterium solanacearum* plated from wilting North Carolina tobacco (the Granville tobacco wilt).

The common vanilla (*Vanilla planifolia* Andrews) also contracted the disease. Under our hothouse conditions it was possible to produce wilt and brownrot only of the softer growing tips of the shoots (Pl. 65), but the bacteria were traced a considerable distance farther in the vascular system, and it seems likely that under tropical conditions whole plants might be subject to the disease. A few plants only having been inoculated in 1918 (always by delicate needle pricks), the inoculations were repeated in 1920 with the same results, using *Bacterium solanacearum* obtained from North Carolina tobacco. These results were wilting and rot of the terminal shoots in about a week or 10 days' time, brown to black stain in the tissues, with multiplication of the bacteria in the vessels (and in the parenchyma of the softer parts); and from the interior of one of these stems a pure culture of the right organism was reisolated.

Sunflowers (*Helianthus annuus* L.) inoculated in the soft stems when about one-fourth grown proved very susceptible. The culture used for this purpose was a subculture from a poured-plate colony isolated from diseased vanilla which was infected from a poured-plate single colony subculture from a diseased tomato which had been inoculated in the same way from a diseased Ricinus. The plants wilted in a week or two with the usual brown staining of the vascular system, and enormous numbers of the bacteria developed in the vascular system to long distances from the point of inoculation, which was near the top of the plant, so that when cross sections of such stems were made the bacteria oozed out copiously (Pl. 59, B), as a gray white slime even down to the surface of the ground. Here also there was a profound dwarfing. The Ricinus organism was reisolated from an inoculated diseased sunflower on Petri dish agar poured plates, and with a subculture from one of the colonies tomatoes were again successfully inoculated (Pl. 66).

[1] BRYAN, Mary K. A NASTURTIUM WILT CAUSED BY BACTERIUM SOLANACEARUM. *In* Jour. Agr. Research, v. 4, no. 5, p. 451. 1915.

Stanford and Wolf [1] having reported successful inoculations of *Bacterium solanacearum* on the common garden balsam (*Impatiens balsamina* L.), we tried the Ricinus organism on these also with marked success, the tops wilting and the brown streaks, due to stained vascular bundles, showing through the translucent stems for long distances down, the inoculation being made near the top (Pl. 67, A). In such cases a microscopic examination showed the vascular system to be filled and honeycombed by the bacteria.

Sections of Ricinus stems were cut and stained for location of the bacteria and disintegration of the tissues, which, corresponding to the slow progress of the disease in our inoculated plants, was not very extensive and not unlike figures already published by the senior writer for other plants attacked by *Bacterium solanacearum*.

Since good figures of the appearance of *Bacterium solanacearum* on agar poured plates are not very numerous, some colonies are shown enlarged 10 times (Pl. 67, B). The surface colonies on nutrient agar poured plates are irregularly roundish, white and shining by reflected light, and very fluid, so that they flow readily when placed in a vertical position. By transmitted light the colonies of this organism are brownish. By oblique light they are opalescent, the play of mother of pearl colors being usually very conspicuous. The organism is strongly aerobic, as is shown by the small buried colonies on the poured plates, feeble growth in the depths of stab cultures, and in various other ways already described. The Ricinus organism when grown on cylinders of sterile steamed potato produced exactly the same brown stain as *Bact. solanacearum* from other plants, and its reaction in milk and litmus milk is also the same.

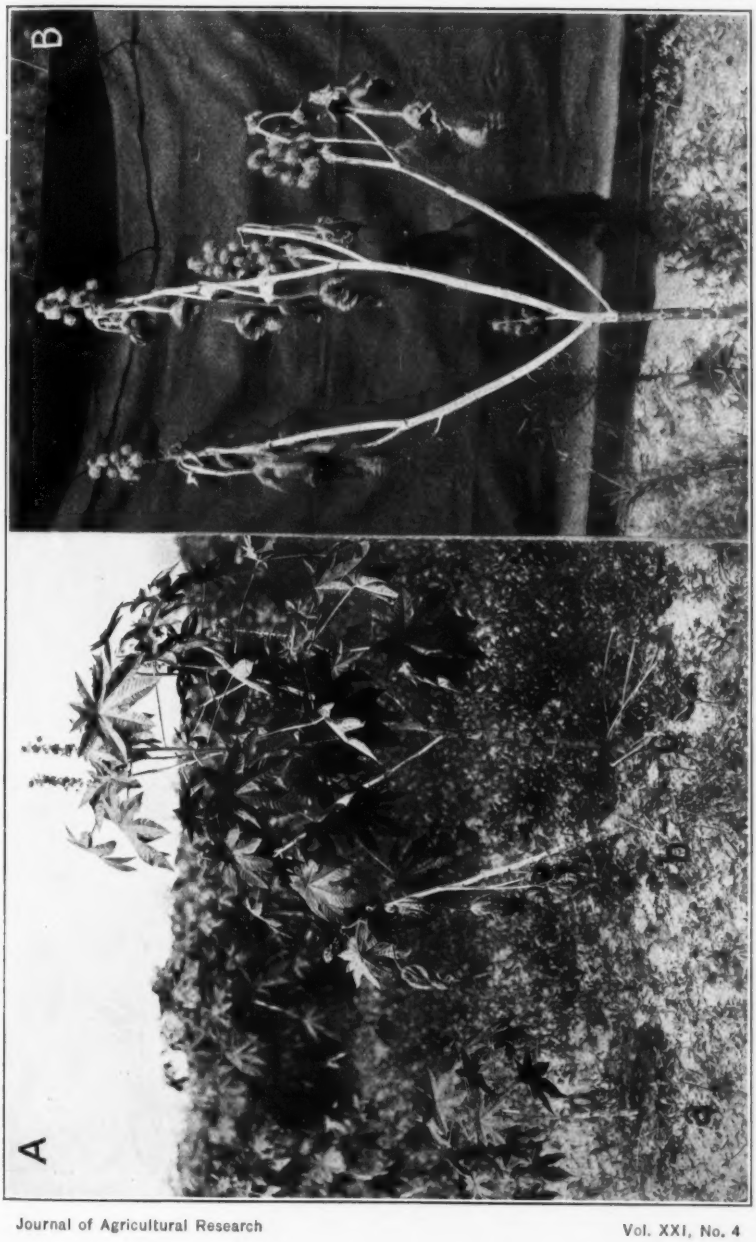
The profound dwarfing of Ricinus plants was obtained again in 1920 with *Bacterium solanacearum* plated from the wilted North Carolina tobacco. In 1920 fuchsias were also shown to be susceptible.

[1] STANFORD, E. E., and WOLF, F. A. STUDIES ON BACTERIUM SOLANACEARUM. *In* Phytopathology, v. 7, no. 3, p. 155–165, 1 fig. Literature cited, p. 165.

PLATE 55

A.—Ricinus plants in Dothan, Ala.: *a*, dwarfed; *b*, wilting,: *c*, healthy. Photographed August, 1918.

B. Fruiting Ricinus plant, badly wilted, at Tampa, Fla.


B
A

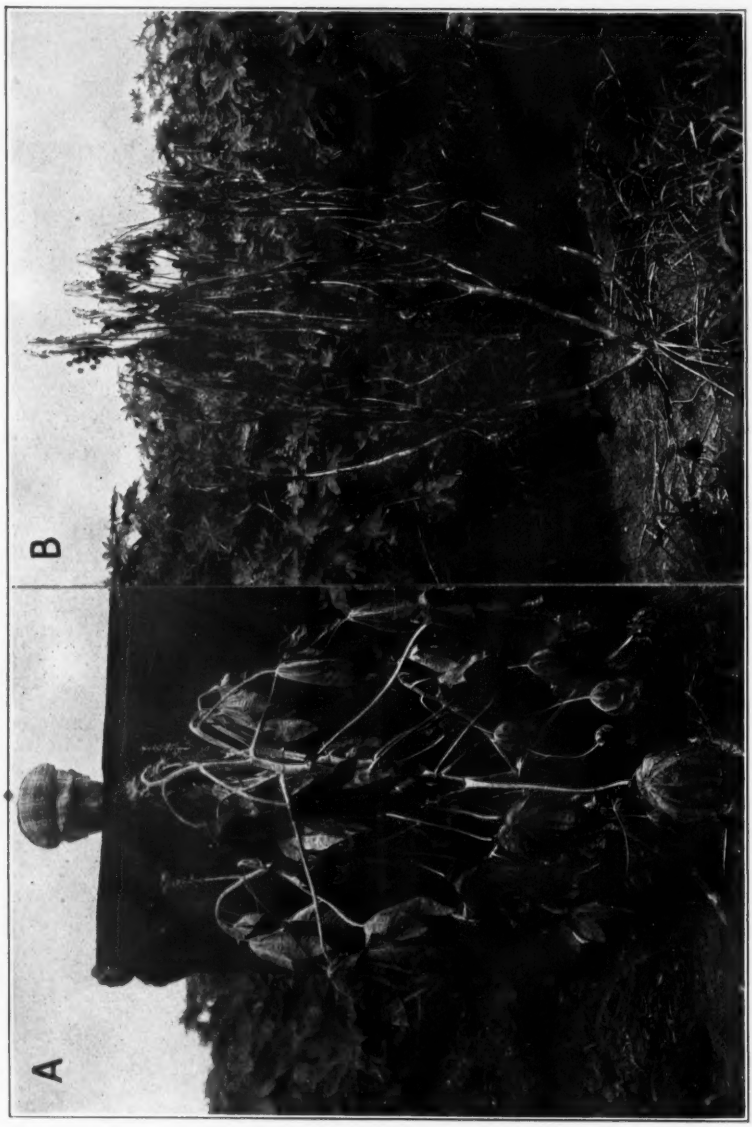

A
B

PLATE 56

A.—Root disease of Ricinus at Miami, Fla., not due to *Bacterium solanacearum*. Water table near surface.

B.—Later ttage of the same disease as in A. Healthy Ricinus plants in the background.

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PLATE 57

Effect of Ricinus wilt organism when inoculated by needle pricks at *XX* into shoots of tomato, June 26, with subculture of a colony isolated from wilted tomato No. 1, which was inoculated from subculture, of a poured-plate colony out of a wilted Ricinus plant received from Townsend, Ga. Photographed July 2, 1918. Much reduced.

X
X

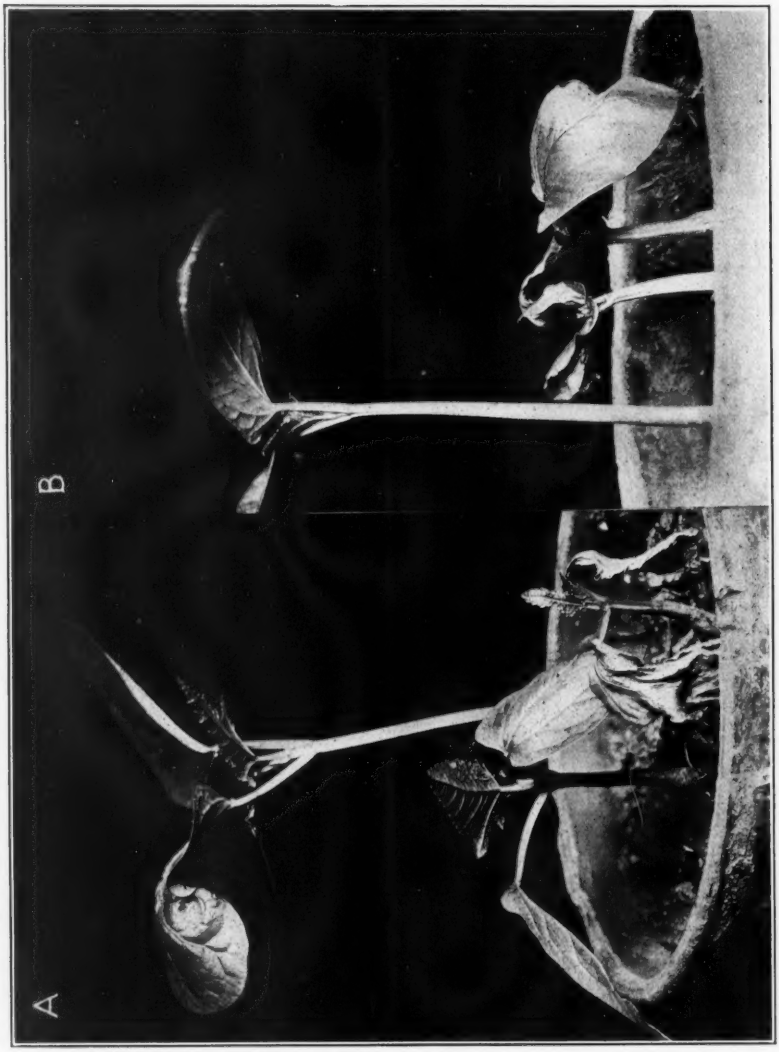

B
A

PLATE 58

A, B.—First four seedling plants to become diseased when inoculated with the Ricinus parasite. Normal control plants in the background. All the inoculated plants are badly dwarfed, but one is not yet wilting. All were inoculated June 26 by needle pricks on the base of the hypocotyl just as the seedling was emerging from the soil. Photographed July 8, 1918. In B the bacteria had penetrated into the vessels of the tap root 2½ inches from the point of inoculation.

PLATE 59

A.—First to wilt of 12 Ricinus plants inoculated July 2 at *X X* with 24-hour potato subculture from inoculated tomato No. 1. This plant when photographed was 6 inches high, while the control plants were 18 inches high. The other 11 inoculated plants were as badly dwarfed as this one, and all of them finally developed wilt. Bacteria were abundant in the vessels of the stem. Photographed July 25, 1918, nearly natural size.

B.—Cross section of middle of stem in an inoculated sunflower, showing the bacterial ooze from the vascular system. × 5.

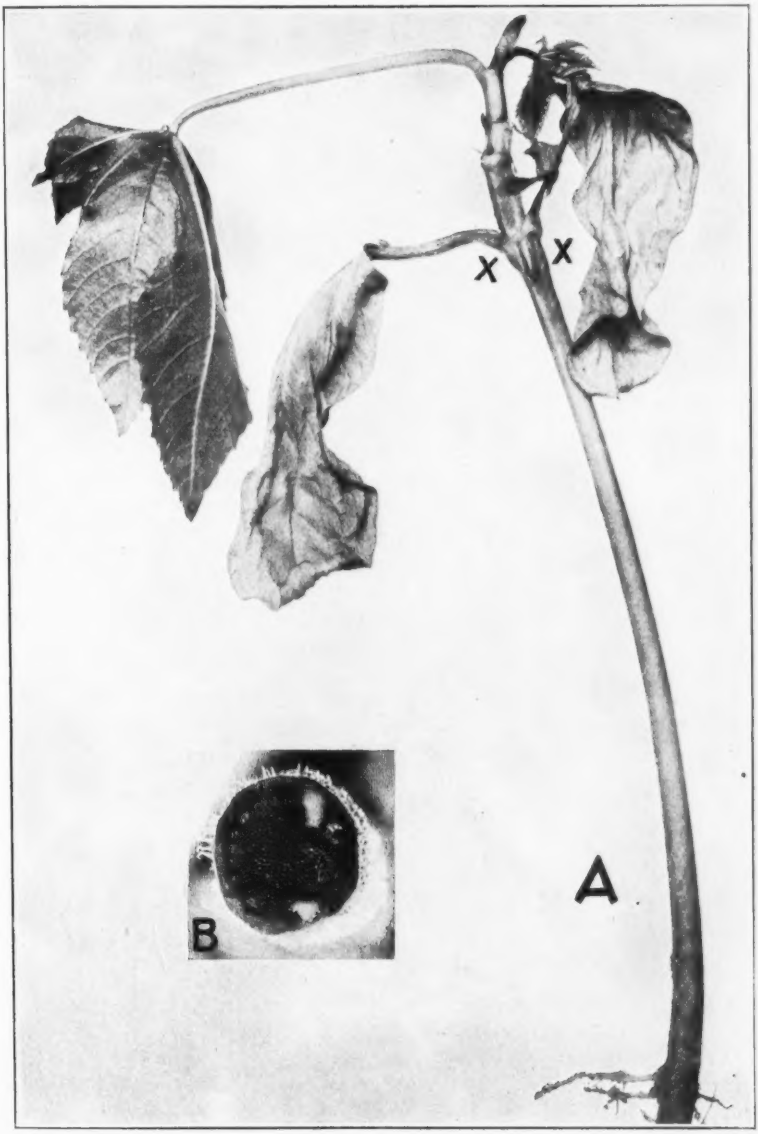

X
X
A
B

PLATE 60

Two plants showing Ricinus wilt, with two controls of the same age in the background. The wilting plants were inoculated July 2 by needle pricks in the top of the hypocotyl with 24-hour potato subculture from poured-plate colony from tomato No. 1. Introduced to show the profound dwarfing. The diseased plants were 7 inches tall and the controls 22 inches. Photographed July 30, 1918.

PLATE 61

First case of wilt in the top of a large blossoming Ricinus plant. This was inoculated by needle pricks at *X X*, on the stem and petiole, June 28, with a culture isolated from tomato No. 1. Bacteria had penetrated into all the main ribs of the blade of the wilted leaf. The brown stain and bacterial occupation of the bundles of the petiole were also very conspicuous. Photographed July 13, 1918.

X
X

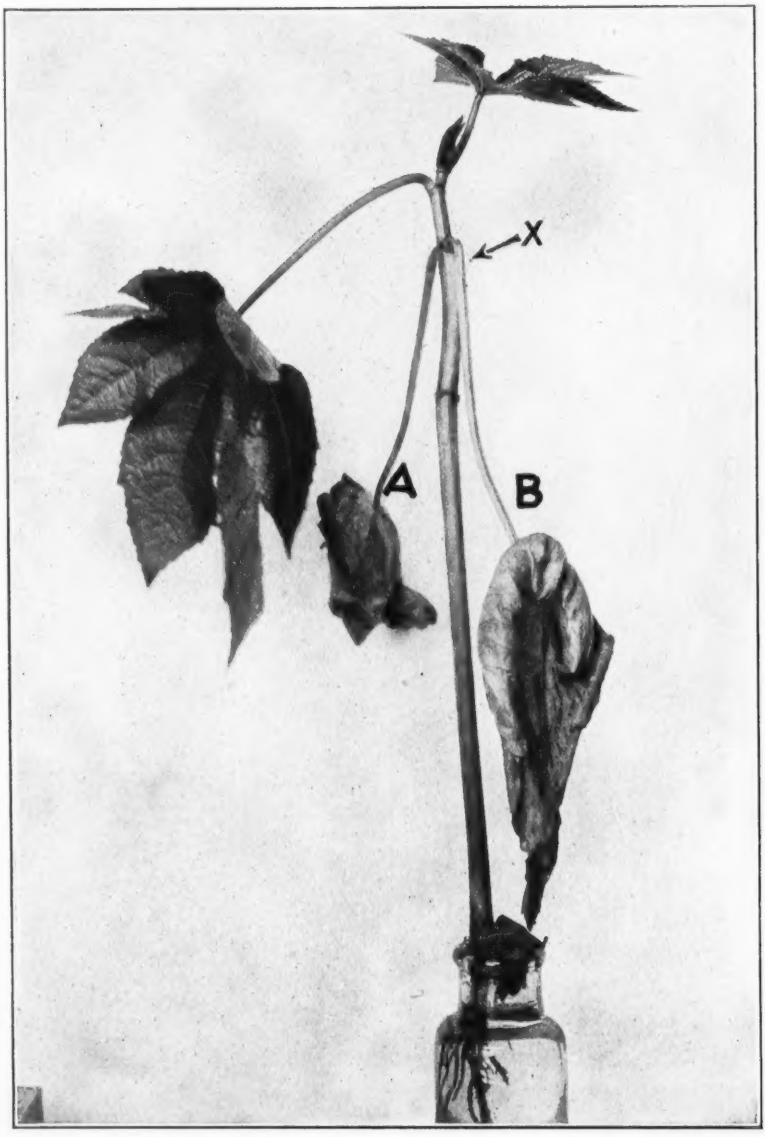

X
A
B

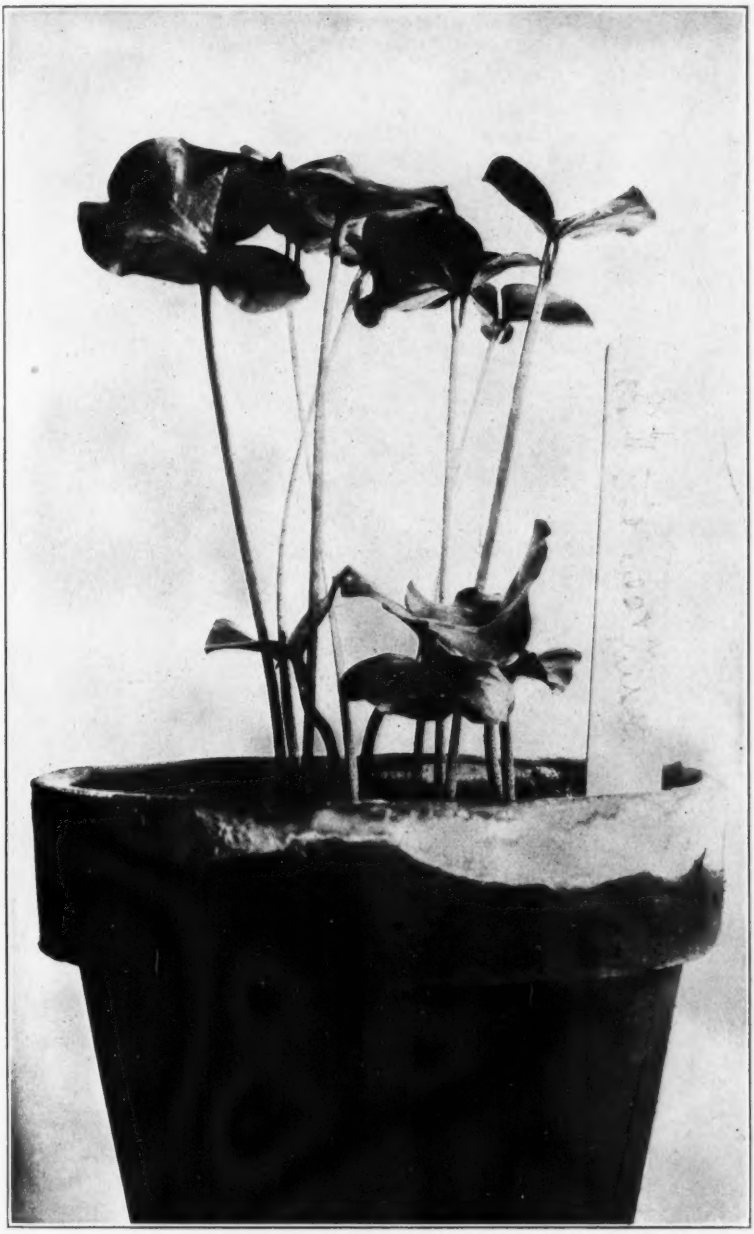
PLATE 62

Plant showing Ricinus wilt infection from seven broken roots. This was one of the control plants shown in figure 127 of "An Introduction to Bacterial Diseases of Plants."[1] The inoculated dwarfed plant was cut and buried in the soil July 17, at which time the roots of the experimental plant were broken. When the plant was photographed the bacteria were most abundant in the roots but were present in the stem as far up as *X*. They were not in the three reflexed petioles, nor in the unbroken roots. Leaves A and B became reflexed before they wilted. Reflexing of tomato petioles in this disease is very common. Photographed July 29. × ½.

[1] Smith, Erwin F. AN INTRODUCTION TO BACTERIAL DISEASES OF PLANTS. XXX, 688 pages, illus. Philadelphia and London. 1920.

PLATE 63

Dwarfing effect of *Bacterium solanacearum* when inoculated into hypocotyls of seedling cotton, variety Egyptian. Controls in the background. The inoculation was by needle pricks, and the plants were all of the same size at that time. The organism was obtained from North Carolina tobacco. A few days later all the inoculated plants wilted and shriveled, at which time they were full of the parasite. Older plants did not take the disease. Photographed July 26, 1920. Time from inoculation, 5 days. For later stages of the disease obtained with the Ricinus wilt organism see Plate 64.



A
B

PLATE 64

Ricinus wilt on seedling cotton. Controls at left. Plants inoculated in the hypocotyl by needle pricks, July 2, 1918, from inoculated tomato No. 1. Plants badly dwarfed and bacteria very abundant in the tissues. Photographed (A) July 15, 1918; (B) July 13, 1918. Each was inoculated with subculture from a poured-plate colony, and the controls were of the same size as the others when the inoculations were made.

PLATE 65

Ricinus wilt organism on *Vanilla planifolia*, inoculated on June 28, 1918, with subculture from colony 2, plate 4, June 19, out of tomato No. 1, the inoculum for which came from the Ricinus received from Townsend, Ga. Photographed July 6, 1918.

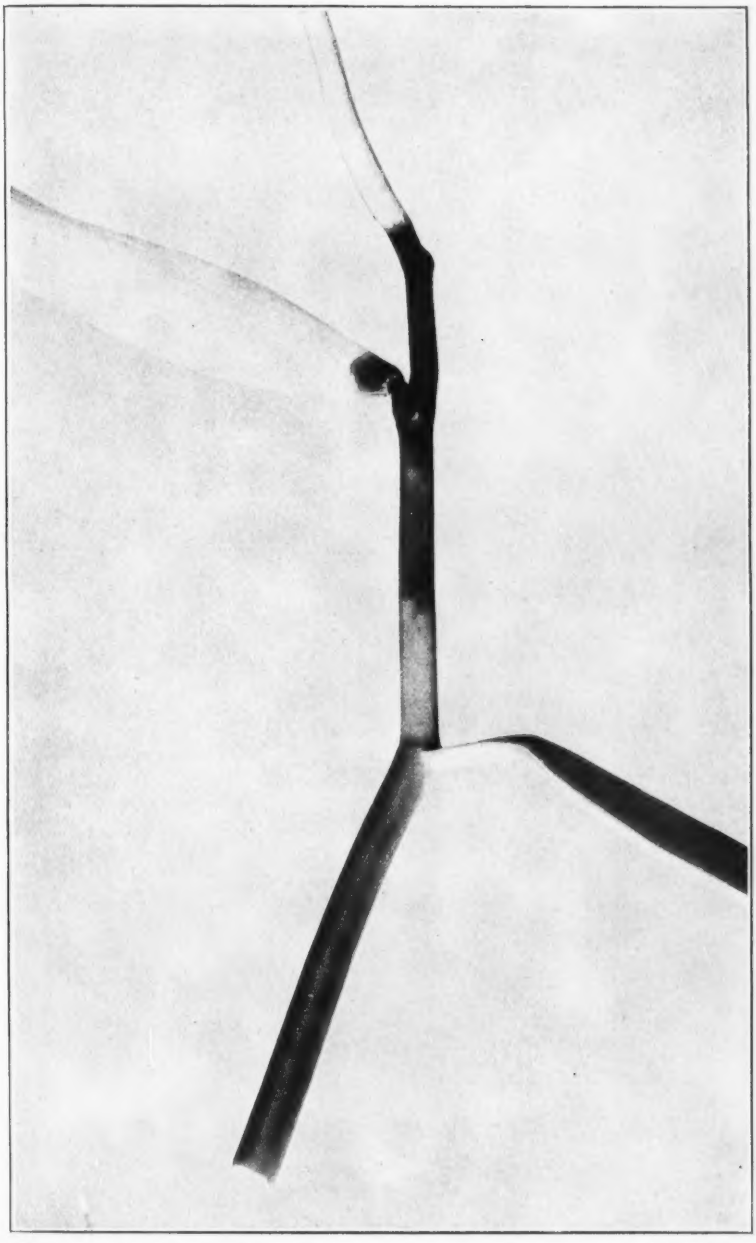


PLATE 66

Another younger tomato plant showing effect of the Ricinus parasite when inoculated by needle pricks. Control plant on right. The plant on the left (dead to the ground on August 7) was inoculated July 24 with a subculture from colony No. 1, plated from inoculated wilting sunflower No. 1. Dwarfing conspicuous; roots present on the stem. Photographed July 30, 1918. × ¼.

PLATE 67

A.—Two stems of the common garden balsam (*Impatiens balsamina*), showing internal brown striping due to the Ricinus wilt organism. The plants were inoculated June 26, 1918, and the photograph was made July 11. The bacteria were extremely abundant in the vessels of these stems. Surface unbroken.

B.—Agar poured-plate colonies of Ricinus wilt orgnism, photographed vertically and introduced to show fluid nature of the colonies and also the strongly aerobic nature of the organism, as may be seen by the small size of the buried colonies as contrasted with the two surface colonies. The colonies flow when tilted, are smooth and glistening on the surface, white by reflected light, pale brown by direct transmitted light, and opalescent by oblique light. Plate 4 days old at 26° C. It makes no difference in fluidity of the colonies whether Witte's peptone is used in the agar or Difco peptone. The opalescence by oblique light is usually very striking. Photographed June 17, 1918. × 10.

A
B

STEWART'S DISEASE OF CORN

By FREDERICK V. RAND, *Pathologist*, and LILLIAN C. CASH, *Scientific Assistant*, *Laboratory of Plant Pathology, Bureau of Plant Industry, United States Department of Agriculture*

The authors have found Stewart's disease (wilt) of corn in Georgia, South Carolina, Tennessee, Virginia, Kentucky, Missouri, Iowa, Illinois, Indiana, Ohio, Pennsylvania, District of Columbia, Maryland, Delaware, New Jersey, southern New York, and Connecticut. Although in the field the number of diseased plants has usually been under 20 per cent, as high as 100 per cent infection has sometimes been found among the earlier varieties. Cultures of the causal organism (*Aplanobacter stewarti* [E F S] McCul.) have been obtained from most of the localities where the disease has been observed by the writers. The disease was not found in Minnesota, Wisconsin, Michigan, northern New York, Vermont, New Hampshire, and Maine. However, it has been reported by other pathologists from Massachusetts, West Virginia, Michigan, Oklahoma, New Mexico, and California.

Seed of 53 varieties of sweetcorn purchased in the open market has been planted during three different seasons in Maryland. As one of the results of this field study it has been found that an arrangement of varieties according to time of maturity coincides almost exactly with an arrangement according to percentage of wilt development. The later varieties such as Zig Zag Evergreen, Stowell's Evergreen, and Country Gentleman have consistently given a very low percentage of the disease (average below 10 per cent), while the earliest varieties under the same conditions have shown a serious loss from wilt (average 25 to 57 per cent). In general, wilt prevalence among midseason varieties has been between these two extremes. First of All has shown the greatest injury, some plantings have given 100 per cent infected stalks, while the Cory group, Golden Bantam, and others of the earliest sorts approach it in susceptibility. The Evergreen group, as a whole, was little affected; and it is interesting to note that Bantam-Evergreen—a cross between Golden Bantam and Stowell's Evergreen—appeared to carry with it none of the susceptibility of the Bantam parent. Among 45 varieties of field corn planted during the same three seasons, 32 have at no time shown traces of wilt. A few of the dent corns have given 5 per cent or less, but it is the earlier flint sorts that have been found most susceptible. For example, Will's Gehu has given as high as 65 per cent of infected stalks; Square Flint, 40; Longfellow, 22; and King Philip, 19.

During the past two seasons six experiments relative to soil transmission have been carried out under field conditions. In some cases the

soil was inoculated with virulent tap water suspensions of the wilt bacteria; in other instances pieces of diseased stalks were thickly strewn in furrows, and the seed of susceptible varieties was planted among and directly over them. No evidence whatever of infection from the soil or from proximity to diseased stalks has thus far been obtained. However, the organism has been isolated from the endosperm of seeds developed on diseased plants. Furthermore, seed collected from known diseased plants gave a higher percentage of wilt than seed of the same varieties purchased in the open market.

Infection of the young corn plant from the seed was found to be largely dependent upon the growth condition of the seedling during the first week or two after planting, as influenced by soil moisture, soil texture and fertility, and temperature. Data relative to seven different plantings during three seasons (1918–1920) in our experimental fields in Maryland and two plantings during 1920 in Maine have shown that the most important single factor predisposing to infection from the seed is soil moisture. Whenever rains have been plentiful about the time of sowing the seed, wilt has later developed in abundance, whereas the same lots of seed planted during a dry period have invariably given much less infection. With moisture conditions approximately the same, the later plantings at higher temperatures have given a greater amount of wilt. Similar relations to temperature were found with inoculations in greenhouse compartments held at different temperatures. Both plantings in Maine, under conditions of drouth and low temperature, gave no wilt at all. On the other hand, the same lots of seed planted at three different dates in Maryland under more favorable conditions of both moisture and temperature gave an abundance of the disease. One planting in Maryland during the same season, although at more favorable temperatures, was sown during a period of drouth, and in this case only 2 per cent of infection occurred. At the same place during 1918 in a 4-acre variety test planted in light sandy soil during an exceptionally dry period only 10 cases of wilt occurred during the whole season. It was further noted that when seed from a single lot was planted simultaneously in light soil, in fertile sandy soil, and in rich sandy loam a considerably higher percentage of infected plants developed in the richer soil. It thus seems apparent that anything which retards the germination and early development of the seedling lessens the chances of infection from the seed. Of these environmental factors soil moisture and temperature seem to be of greatest importance.

Control methods are still in the experimental stage. However, these preliminary results seem to indicate that northern-grown seed is less likely to carry infection than that grown farther south, and that infected seed may be rendered safe for planting by a dry heat "pasteurization" at 60° to 70° C. for one hour.

QUALITY OF IRRIGATION WATER IN RELATION TO LAND RECLAMATION

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The so-called alkali problem on irrigated lands has two distinct phases. In one case the difficulty is due to the fact that, because of inadequate drainage or because of excessive quantities of salt in the irrigation water, the salt content of the soil solution comes to exceed the limit of tolerance of crop plants. In the other case the soil becomes relatively impermeable to water, difficult to work into good tilth after wetting, and in some cases nearly or quite unproductive. The present paper deals with this second phase of the alkali problem.

These impermeable soils or hard lands are of wide occurrence in the irrigated regions. The condition may be found in desert soils that have not been irrigated, or it may develop after a period of irrigation. It is of very common though not universal occurrence in lands that have been swamped through the rise of the ground water and later reclaimed by drainage. The condition of impermeability or hardness is one of degree, some lands though hard being much more permeable than others. It is rather uncommon to find land that is completely impermeable to irrigation water.

These hard lands sometimes contain soluble salts in excess of the generally recognized limits for crop production, but more often this is not the case. Very salty land may take water well and be soft or even "puffy" on the surface when dry, but when reclaimed by drainage and irrigation it may become so hard as to be very difficult to irrigate and difficult to work into good tilth when dry.

The subject of the slow permeability of soils has been extensively investigated, both in this country and in Europe. The condition is not confined to desert or irrigated land but is found also in many regions where the rainfall is abundant. In the latter case it is manifested either by apparent infertility or by surface erosion of the soil or by both conditions. It is a matter of common observation that the erosion of soil by rainfall is more directly associated with conditions of slow permeability to water than with conditions of topography. In other words, with a given slope a soil that is readily permeable to water is much less subject to erosion by rainfall than a soil that takes water slowly.

Journal of Agricultural Research,
Washington, D. C.
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Vol. XXI, No. 4
May 16, 1921
Key No. G-234

Where this problem has been investigated on desert or irrigated land it has been the consensus of opinion that the condition is due to, or is at least associated with, the occurrence of sodium carbonate or "black alkali" in the soil solution. As a result of the present investigation we have reached the conclusion that the trouble is not due to sodium carbonate alone but that it may be induced by the so-called neutral salts of sodium as well.

It is well known that if a soil containing clay is leached or irrigated with water containing sodium carbonate, even in very dilute solution, the rate of percolation will become greatly retarded. Sharp [1] observed a similar retardation in the rate of percolation as a result of leaching with pure water soils to which sodium chlorid and sodium sulphate had been added. We have found that when a soil treated with sodium chlorid or sodium sulphate is leached with pure water or with solutions of these salts much more dilute than the soil solution, the rate of percolation is retarded and the percolate shows a strongly alkaline reaction. In other words, in leaching from the soil the neutral salts of sodium the permeability of the soil is reduced and the soil solution is made alkaline. These are the characteristic features of land that becomes "hard" after a period of irrigation and of salty land that has been reclaimed by drainage.

We have also observed in these investigations that this injurious effect of the salts of sodium is much reduced if the salty soil is first leached with a solution of some salt of calcium or aluminum. A soil so treated may subsequently be leached with pure water without developing the symptoms of hard land, namely, reduced permeability and an alkaline percolate. Similarly we have found that if the irrigation water contains calcium salts equal to or exceeding in quantity the salts of sodium, the soil rendered salty by such water is less likely to become impermeable when subsequently leached with pure water or with water containing these salts in the same proportion but in lower concentration.

These observations lead us to the conclusion that the quality of irrigation waters should be judged not only by considering the total quantity of the salts in solution or the proportions of the acid radicles, but also the proportion of the sodium to the calcium and magnesium. It is suggested that water, to be safe for long-continued irrigation, should be relatively rich in calcium and magnesium.

In colloquial language, waters containing relatively large quantities of calcium and magnesium are known as "hard" waters. The results of the present investigation may be summarized in the simple statement: Hard water makes soft land and soft water makes hard land.

[1] SHARP, L. T. FUNDAMENTAL INTERRELATIONSHIPS BETWEEN CERTAIN SOLUBLE SALTS AND COLLOIDS. In Univ. Calif. Pub. Agr. Sci., v. 1, no. 10, p. 291–339, 3 fig. 1916. Bibliographical footnotes.

THEORY OF THE REACTIONS

The reactions that take place in a soil that is leached with a dilute salt solution were first investigated in detail by Way.[1] Later investigators have dealt with various aspects of this subject, but too often without distinguishing clearly between the chemical and physical reactions involved. It was shown by Way that when a solution of a salt was leached through a soil containing clay a chemical reaction took place as a result of which some of the basic element of the salt was retained in the soil and an equivalent quantity of other bases was released into the soil solution and appeared in the percolate. Thus, if a solution of ammonium sulphate, sodium sulphate, or potassium sulphate was leached through a soil a portion of the base was retained by the soil and the other bases, chiefly calcium, passed into solution. It was definitely pointed out that these chemical reactions were essentially different from the physical property of soils known as adsorption which is common also to other finely divided substances such as charcoal and carbon black.

The reactions reported in the present paper appear to belong to the same category as those discovered by Way. There is, however, an additional fact to be noted. In the present investigation the question under consideration was not primarily the changes taking place in the soil solution but the changes in the physical character of a soil, both with respect to its permeability to water and to its characteristics upon drying. It has been found that leaching sodium salts through a soil causes it to become less permeable to subsequent leachings with water, and, furthermore, upon drying the soil becomes hard, a condition familiarly described in agriculture as "baking." Calcium salts, on the other hand, have been found to render the soil readily permeable for water and less likely to become hard or "baked" upon drying.

It has been shown by experiments on small samples, not here reported in detail, that the soluble salts of aluminum, such as chlorids and sulphates, are much more effective than the similar salts of calcium in improving the physical condition of impermeable soils. Soluble aluminum salts, such as aluminum sulphate, if leached through a sample even of extremely refractory soil, increases its permeability for water and completely removes the tendency to become hard on drying, the soil remaining very soft and powdery. Furthermore, these characteristics persist even after repeated leachings with distilled water.

The theory advanced to explain these facts is that the different bases in the solutions used partially displace the bases in the compounds on the surface of the soil particles. For example, when a solution of a salt of sodium is leached through a soil, the sodium will displace a portion

[1] WAY, J. THOMAS. ON THE POWER OF SOILS TO ABSORB MANURE. *In* Jour. Roy. Agr. Soc. England, v. 11, p. 313-379. 1850; v. 13, p. 123-143. 1852.

[2] ——— ON THE INFLUENCE OF LIME ON THE "ABSORPTIVE PROPERTIES" OF SOILS. *In* Jour. Roy. Agr. Soc. England, v. 15, p. 491-514. 1854.

of the bases on the surface of the soil particles, itself becoming combined and remaining on the soil particles, partly at least, in the form of a silicate. This silicate, being in the form of a colloid, a hydrogel, will increase the effective size of each soil particle and thus greatly retard the movement of water through the soil and may, in fact, stop percolation entirely. Furthermore, on drying, this colloidal gel will tend to bind the soil particles in a solid mass. With solutions of calcium or aluminum, on the other hand, when the displacement of bases on the surfaces of the soil particles takes place colloid gels are not formed, and, in fact, such sodium silicate hydrogels as may exist there are almost completely eliminated or masked by the formation of practically insoluble precipitates of calcium or aluminum.

From these preliminary experiments it is also evident that the salts of aluminum, like the salts of calcium, when applied to the soil release into the soil solution other bases that may be present but not in solution. It is assumed that such bases as ammonia, sodium, potassium, calcium, and magnesium are displaced by the aluminum from their combination in the silicates of the soil and unite with the acid radical of the aluminum salt. If this assumption is correct, it would naturally follow that soluble salts of aluminum may be useful both in improving the physical condition of a soil and also in rendering other bases more soluble in the soil solution and presumably more available for the nutrition of plants.

EFFECT OF SODIUM SALTS

The injurious effects resulting from leaching a neutral sodium salt from the soil may be demonstrated by a very simple experiment. If a sample of a permeable soil be placed in a funnel over a filter paper or in an ordinary laboratory percolation tube, its percolation rate with water may be determined. If the soil is then leached with a solution of sodium chlorid or of sodium sulphate or a mixture of the two salts in constant or in increasing concentrations the rate of percolation will remain approximately constant, or it may be increased slightly over that observed with pure water and the percolate will continue clear and nearly or quite neutral in reaction. On the other hand, if the concentration of the leaching solution is reduced or if the soil is leached with pure water after treatment with the salt solutions three phenomena develop, namely, the rate of percolation is reduced, the percolate becomes turbid, and it also becomes strongly alkaline.

If the soil so treated is rich in clay, these phenomena are developed to a marked degree. With some clay soils the effect is so pronounced that it is not possible to collect a sufficient quantity of the percolate for analysis. The course of events just described is illustrated in Table I. In this case the soil used was a sample of mesa sand from the Yuma Mesa in southwestern Arizona. It was taken from a point on the mesa which had not been irrigated. The proportion of clay was very small,

and the soil was rich in calcium carbonate. In this experiment 300 gm. of dry soil were placed in the percolation tube and leached first with distilled water, 100 cc. being used for each leaching. After seven leachings with water, solutions of sodium chlorid were added, each successive solution being stronger than the preceding, until at the twentieth leaching a concentration of 5 per cent was reached. For the twenty-fifth leaching a solution of 3.7 per cent was used, and the twenty-sixth and later leachings were made with pure water.

Table I shows the concentration of the various salt solutions used, the rate of percolation, the concentration of the later percolates as shown by the electrolytic bridge, the chlorids in the percolate as determined by titration with silver nitrate and calculated as sodium chlorid,[1] the alkalinity of the percolate as determinated by titration with *N/10* sulphuric acid, using butter yellow as an indicator, and the character of the percolate with respect to turbidity.

TABLE I.—*Effect of leaching with sodium-chlorid solution followed by water on the rate of percolation and the character of the percolate*

Leaching No.	Leaching solution.	Percolation rate per minute.	Salts in percolate (by bridge).	Chlorids as NaCl.	Acid *N/10* H_2SO_4 for 100 cc. percolate.	Character of percolate.
		Cc.	*P. p. m.*	*P. p. m.*	*Cc.*	
1	Water	1.25	.....	58	1.80	Yellowish.
2	.....do	1.75	.....	23	1.40	Whitish turbid.
3	.....do	1.50	.....	15	.80	Do.
4	.....do	1.64	.....	47	.66	Do.
5	.....do	2.13	.....	35	.60	Do.
6	.....do	2.00	.....	23	.66	Do.
7	.....do	.....	.....	18	.66	Do.
8	NaCl p. p. m. 93	1.54	.....	70	.66	Slightly turbid.
9	NaCl p. p. m. 210	1.82	.....	164	.32	Clear.
10	NaCl p. p. m. 327	.....	.....	281	.26	Do.
11	NaCl p. p. m. 421	1.67	.....	375	.32	Do.
12	NaCl p. p. m. 515	1.70	.....	468	.32	Do.
13	NaCl p. p. m. 795	.....	.....	643	.34	Do.
14	NaCl p. p. m. 1,028.	1.23	.....	643	1.00	Do.
15	NaCl p. p. m. 1,987.	2.03	.....	1,286	.60	Do.
16	NaCl p. p. m. 3,226.	1.89	.....	2,457	.40	Do.
17	NaCl p. p. m. 5,150.	2.00	.....	3,510	.50	Do.
18	NaCl p. p. m. 10,520.	1.70	.....	7,030	.30	Do.
19	NaCl p. p. m. 25,000.	1.67	.....	15,780	.40	Do.
20	NaCl p. p. m. 50,000.	.....	.....	32,750	.60	Do.
21	NaCl p. p. m. 50,000.	1.43	.....	65,520	1.20	Do.
22	NaCl p. p. m. 50,000.	1.33	.....	47,969	1.30	Do.
23	NaCl p. p. m. 50,000.	1.25	.....	49,725	1.10	Do.

[1] The clear percolates, particularly those resulting from the use of the strong salt solutions, showed the presence of calcium, but the quantity was not determinated.

TABLE I.—*Effect of leaching with sodium-chlorid solution followed by water on the rate of percolation and the character of the percolate*—Continued

Leaching No.	Leaching solution.	Percolation rate per minute.	Salts in percolate (by bridge).	Chlorids as NaCl.	Acid $N/10$ H_2SO_4 for 100 cc. percolate.	Character of percolate.
		Cc.	*P. p. m.*	*P. p. m.*	*Cc.*	
24	NaCl p. p. m. 50,000.	1.00	.....	48,555	1.10	Clear.
25	NaCl p. p. m. 37,440.	.....	.....	46,780	1.20	Do.
26a	Water.....	[a].364	.....	32,770	1.40	Do.
26b	.....do.....	[b].235	.....	40,014	1.50	Very turbid.
26c	.....do.....	[c].316	1,930	562	19.20	Do.
27	.....do.....	.267	1,370	281	14.60	Do.
28	.....do.....	.....	780	199	12.40	Do.
29	.....do.....	.....	622	47	11.00	Do.
30	.....do.....	.....	569	Trace.	6.40	Turbid.
31	.....do.....	.154	506	...do....	5.90	Do.
32	.....do.....	.....	486	None.	6.70	Do.
33	.....do.....	.160	450	...do....	6.60	Do.
34	.....do.....	.142	371	...do....	4.60	Do.
35	.....do.....	.132	371	...do....	4.00	Do.
36	.....do.....	.154	350	...do....	5.60	Do.
37	.....do.....	.140	314	...do....	3.40	Do.
38	.....do.....	.131	294	...do....	4.10	Do.
39	.....do.....	.091	258	...do....	3.80	Do.
40	.....do.....	.127	233	...do....	3.04	Do.
41	.....do.....	.800	195	...do....	3.40	Do.
42	.....do.....	.760	133	...do....	2.40	Do.
43	.....do.....	.530	132	...do....	1.40	Do.

[a] 40 cc. [b] 20 cc. [c] 20 cc.

It will be observed from Table I that the percolates from the first distilled water leachings showed a slight whitish turbidity. This disappeared after the leachings with the salt solutions were begun, but the percolate became very turbid when water was again used after the salt solutions. The first distilled water leachings gave some alkalinity in the percolates; but this became less and continued very slight, with one exception, until the strongest salt solutions were used. With leaching No. 26, the first with distilled water after the salt solutions, a marked change took place. The first part of the percolate from this leaching differed little from the preceding, but the latter part was very turbid and strongly alkaline. The exception as to alkalinity noted above is seen in percolate No. 14. This may be explained by the fact that leaching No. 13 was made on Saturday and No. 14 was made on the following Monday. Although the salt solution used for No. 14 was somewhat stronger than the preceding, evaporation from the tube had been sufficient, apparently, to make the concentration of the soil solution stronger than the concentration of the leaching solution. Similar results have been noted where leachings have been made with solutions of constant strength but with intervals between the successive leachings during which the soil was allowed to dry out.

It will be noted from Table I that the alkalinity and turbidity of the percolate continued through many leachings after the use of distilled water was resumed, while the chlorids quickly disappeared. The rate of percolation also declined sharply when the leaching with distilled water was resumed.

It is probable that the course of events indicated in part in the experiment just described may be explained as a partial change of the bases in the silicates of the clay in the soil. It is assumed that some of the sodium displaces some of the calcium in these silicates and that the calcium passes out as calcium chlorid. The resultant sodium silicates remain insoluble in the presence of such strong acid radicles as the sulphates and chlorids, but as these are leached away the silicates become soluble and by their high viscosity retard the rate of percolation.

A similar substitution of bases is well known and widely used in the so-called zeolite process of water softening. In this process an artificial zeolite, rich in sodium, is used. The "hard" water to be treated is leached through the zeolite with the result that the lime is absorbed and some of the sodium is given up. From time to time the zeolite is restocked with sodium and the absorbed calcium is replaced by leaching it with a strong salt solution.

It has been shown by Kelley and Cummins [1] that such a substitution of bases takes place in the soil, presumably in the silicates, when soils are digested with dilute salt solutions. They found that calcium is extensively replaced by sodium and that sodium is replaced by calcium, though in the dilutions and with the soils with which they worked the latter reaction was much less marked than the former. The final paragraph of their conclusions is sufficiently significant to warrant quotation.

> It is suggested that the continued addition of soluble salts in the open field where the products of the reactions are removed by either the growth of crops or intermittent leaching must ultimately result in building up a chemical system different from that originally present. As will be shown later, the physical properties of the system also may be materially altered.

EFFECT OF CALCIUM SALTS

It was remarked above that the salts of calcium and of aluminum react differently in relation to subsequent leachings with distilled water than the salts of sodium. The use of gypsum (calcium sulphate) has been widely recommended as a corrective for difficulties with black alkali. These recommendations are usually based on the assumption that calcium sulphate reacts with the sodium carbonate in the soil solution to form calcium carbonate, which is very slightly soluble, and sodium sulphate, which is one of the least toxic of the sodium salts.

It is well established by numerous field experiments that gypsum exerts a beneficial effect when used on hard land. It is not very soluble,

[1] KELLEY, W. P., and CUMMINS, A. B. CHEMICAL EFFECT OF SALTS ON SOILS. *In* Soil Sci., v. 11, no. 2, p. 139–159, 7 fig. 1921. References, p. 158–159.

however, and has not been found effective where the soil is very impermeable and where it is not practicable to mix the salt thoroughly with the soil.

It has been found in these experiments that calcium chlorid is more effective in penetrating a compact and impermeable soil than the weaker solutions of calcium sulphate. It has also proved effective in making such soils readily permeable to water, and this permeability is retained after the calcium chlorid is well leached out with pure water.

In a preceding paragraph it was remarked that the injurious effects of sodium salts in irrigation water were minimized when calcium salts were also present in sufficient quantity. It may not be possible to state definitely what these proportions should be in all cases, as this may be conditioned somewhat by the character of the soil. It is clear, however, that a soil rich in lime is not proof against injury from the sodium salts carried in the irrigation water.

The neutralizing effect of calcium in solution with sodium is shown in Table II. In this experiment the soil used was also from the Yuma Mesa, but from a field that had been irrigated for 25 years with muddy water from the Colorado River, so that it was rich in silt and clay. The sample used consisted of 50 gm. of dry soil placed in a funnel over a small filter paper. It was leached each time with 100 cc. of solution or water. This soil was first leached with a strong solution of calcium chlorid which was followed by distilled water. It was then leached with a solution of equal parts of *M/10* sodium chlorid and *M/20* calcium chlorid, which was again followed by several leachings with water. It was finally leached with a solution containing 75 parts of *M/10* sodium chlorid and 25 parts *M/20* calcium chlorid, followed by distilled water.

The results given in Table II show that the percolation rate for the water leachings was little or not at all retarded when they followed the calcium chlorid or the mixture of equal parts of the chlorids of calcium and sodium. On the other hand, when the proportion of the sodium salt was much greater than that of the calcium the subsequent water leachings showed the same symptoms as when the pure sodium salt was used. In this case the symptoms were rather more pronounced than with the soil used for the leachings shown in Table I, probably because of the larger proportion of clay in the soil.

From these results it seems probable that when irrigation water contains much more sodium than calcium its use may be followed by an appreciable hardening of the soil. Where the difference in the quantity of the two salts is not great or where the total quantity of the sodium is not large it may be practicable to prevent injury by the use of gypsum on the land.

In order to test the effects of sodium and calcium salts on a soil that was known to be naturally almost impermeable, a sample was taken from a field on the Newlands project near Fallon, Nev. This soil is so hard and impermeable that it is practically unproductive, yet when it is

dry and thoroughly pulverized it has the appearance of a sandy soil. In pot cultures, when treated with gypsum and manure, it is very productive, but it has not been found possible to reclaim it completely with gypsum in the field.

The results of leaching experiments with Fallon soil are shown in Tables III and IV. In these experiments 50 gm. of dry soil were leached with 50 cc. of solution followed by the same quantity of distilled water. In one case, Table III, the chlorids of calcium and sodium were compared, and in the other case, Table IV, the sulphates of the same bases were used.

TABLE II.—*Effect of calcium chlorid and of mixtures of calcium chlorid and sodium chlorid when followed by water on the rate of percolation and the character of the percolate*

Leaching No.	Leaching solution.	Percolation rate per minute.	Acid required to neutralize 100 cc. of percolate.	Character of percolate.
		Cc.	*Cc.*	
1	*M*/6.25 $CaCl_2$	2.25	0.8	Clear.
2	Water	2.50	.6	Do.
3	do	1.58	.5	Do.
4	do	1.25	1.0	Do.
5	do	1.92	.8	Do.
6	do	1.41	.8	Do.
7	*M*/10 NaCl 50 cc. + *M*/20 $CaCl_2$ 50 cc.	1.28	.4	Do.
8	Water	2.57	.8	Do.
9	do	2.83	.8	Do.
10	do	2.66	.8	Slightly turbid.
11	do	3.00	.5	Turbid.
12	do	2.78	.7	Do.
13	*M*/10 NaCl 75 cc. + *M*/20 $CaCl_2$ 25 cc.	2.83	.3	Clear.
14	Water	2.59	.4	Turbid.
15	do	.65	.8	Very turbid.
16	do	.62	1.2	Turbid.
17	do	.036	2.8	Do.

TABLE III.—*Comparison of leaching with sodium chlorid and calcium chlorid, followed by water, showing the rate of percolation and the character of the percolate*

Leaching No.	Leaching solution.	Percolation rate per minute.	Acid required to neutralize 100 cc. of percolate.	Character of percolate.
		Cc.	*Cc.*	
1	*M*/10 NaCl	2.78	3.5	Turbid.
2	do	1.35	1.5	Clear.
3	Water	.19	8.5	Very turbid.
4	do	.023	8.5	Do.
5	do	.028	7.5	Do.
1	*M*/20 $CaCl_2$	4.50	2.0	Slightly turbid.
2	do	1.72	1.0	Clear.
3	Water	1.02	1.4	Whitish turbid.
4	do	.67	1.0	Do.
5	do	.50	3.0	Do.

TABLE IV.—*Comparison of leaching with sodium sulphate and calcium sulphate, followed by water, showing the rate of percolation and the character of the percolate*

Leaching No.	Leaching solution.	Percolation rate per minute.	Acid required to neutralize 100 cc. of percolate.	Character of percolate.
		Cc.	*Cc.*	
1	*M*/10 Na_2SO_4	3.33	3.0	Slightly turbid.
2	do	1.92	1.7	Clear.
3	do	1.92	1.5	Do.
4	Water	.62	8.5	Very turbid.
5	do	.007	8.5	Do.
1	$CaSO_4$, Sat. Sol	2.18	3.0	Slightly turbid.
2	do	1.47	1.8	Do.
3	do	1.16	1.8	Clear.
4	Water	1.43	1.5	Do.
5	do	2.17	4.0	Do.
6	do	1.79	2.2	Do.
7	do	1.73	1.5	Do.
8	do	1.56	1.5	Do.

Although the soil used in these experiments is almost impermeable to pure water it is easily leached with fairly strong (*M*/10) solutions of sodium chlorid or sodium sulphate. When these salt solutions are followed with water, however, the rate of percolation is at once greatly reduced and the percolate becomes turbid and alkaline. The same soil is also permeable to solutions of calcium chlorid and calcium sulphate and remains fairly permeable through the subsequent leachings with distilled water.

BASES OF IRRIGATION WATERS

In respect to the reactions produced in the soil it is assumed that potassium reacts in much the same way that sodium does and that magnesium reacts like calcium. For purposes of comparing one stream or water supply with another it seems proper to take the sum of the calcium and the magnesium as one factor and the sum of the sodium and the potassium as the other factor in a proportion. For convenience we may designate the first factor calcium and the second factor sodium and the ratio between them the calcium-sodium ratio.

The calcium-sodium ratio of the average of the analyses of 19 great rivers of the earth as given by Hilgard [1] is 84 to 16—that is, the sum of the calcium and the magnesium is to the sum of the sodium and potassium as 84 is to 16. According to the same authority the calcium-sodium ratio of the Nile is 69 to 31 for the high-water stage and 79 to 21 for the low-water stage. It may be remarked that the calcium-sodium ratio of a stream is a much more constant factor at any given point than is the total salt content.

For purposes of comparison with the ratios given above, data are assembled in Table V concerning 19 important irrigation streams in the

[1] HILGARD, E. W. SOILS . . . p. 23. New York, London. 1906.

western United States. In this table the chief bases are shown in columns 2, 3, and 4, stated in milligrams per liter. In the last column is given the calcium-sodium ratio for each stream. It will be noted that only one of the streams reported in Table V shows as high a proportion of calcium as the Nile, and none is as high as the average reported for the 19 great rivers of the earth.

In the absence of accurate data as to the extent and seriousness of the "hard" lands in the areas irrigated by these streams it is not possible to determine the correlation between the quality of the water and the condition of the land. It may be noted, however, that the land irrigated by the Belle Fourche and North Platte Rivers is notably free from difficulties as to impermeability though the soil at Belle Fourche particularly is very rich in clay. On the other hand, the land irrigated from the Salt and Gila Rivers in Arizona and that irrigated from the Truckee and Carson Rivers in Nevada is notably subject to difficulties of impermeability.

TABLE V.—*Analyses of 19 important irrigation streams in the western United States, showing quantities of calcium, magnesium, and sodium plus three-fourths of the potassium, and the proportion of the sum of the calcium and magnesium to the sum of the sodium plus three-fourths the potassium* [1]

Stream and station.	Milligrams per liter.			Proportion of the sum of the calcium and magnesium to the sum of the sodium plus three-fourths the potassium.
	Calcium.	Magnesium.	Sodium plus three-fourths of the potassium.	
Belle Fourche, Belle Fourche, S. Dak.	130	37	57	75 : 25
Big Horn, Fort Custer, Mont.	63	21	44	66 : 34
Boise, Boise, Idaho.	18	4	16	58 : 42
Carson, Hazen, Nev.	32	8.6	30	57 : 43
Colorado, Yuma, Ariz.	92	23	110	51 : 49
Gila, San Carlos, Ariz.	81	22	150	41 : 59
Grand, Palisade, Colo.	62	17	66	55 : 45
Green, Jensen, Utah.	55	17	45	63 : 37
Link, Klamath Falls, Oreg.	12	6	21	46 : 54
Little Colorado, Woodruff, Ariz.	68	17	110	44 : 56
Milk, Havre, Mont.	47	29	120	39 : 61
North Platte, Fort Laramie, Wyo.	69	18	40	68 : 32
Pecos, Dayton, N. Mex.	440	100	400	57 : 43
Rio Grande, El Paso, Tex.	100	18	110	52 : 48
Sacramento, Red Bluff, Calif.	16	7.4	16	59 : 41
Salt, Roosevelt, Ariz.	59	18	110	41 : 59
Shoshone, Cody, Wyo.	23	6.7	29	51 : 49
Truckee, Derby, Nev.	20	6.9	29	48 : 52
Yellowstone, Billings, Mont.	39	11	36	58 : 42

[1] STABLER, Herman. SOME STREAM WATERS OF THE WESTERN UNITED STATES. . . U. S. Geol. Survey, Water-Supply Paper 274, 188 p. 1911.

In discussing his methods of analysis, Stabler says: "The figure representing sodium and potassium together was obtained by calculating the weight of their combined chlorides to sodium. The result is in reality the amount of sodium plus three-fourths the potassium, and is so reported in the tables."

In view of the fact that the potassium content of most of these waters is low, the difference between this figure and the actual sum of sodium and potassium is probably not great.

The waters of the Colorado River and its tributaries, the Salt and the Gila, are so extensively utilized for irrigation that they merit particular emphasis in the present connection. These waters have been the subject of extensive investigation and careful analysis. The Salt River is reported in Table V, on the basis of samples taken at Roosevelt, Ariz., as showing a calcium-sodium ratio of 41 to 59. From samples collected daily for a year at a point lower down the stream, Forbes has reported in detail as shown in Table VI. The analyses made by Forbes show a higher salt content than those reported from Roosevelt and a higher proportion of sodium. It is clear that the long-continued use of water carrying such a high proportion of sodium must tend to produce impermeability in the land unless corrective measures are used.

The Gila River is characterized by great fluctuations of discharge. It drains a large watershed that is subject to torrential rains that gather into silt-laden floods. The quantity and character of the more important bases carried by this stream are shown in Table VII, which covers the period of a year. It will be noted that the calcium-sodium ratio given for the Gila in Table V corresponds closely with that reported for the summer flood period in Table VII.

The quality of the water of the Colorado River at Yuma is given for seven characteristic periods of a year in Table VIII. It will be noted that the main river at Yuma carries less salt and a higher proportion of calcium and magnesium than its Arizona tributaries. In this it probably reflects the influence of its northern tributaries such as the Green and the Grand shown in Table V.

TABLE VI.—*Analysis of Salt River water samples taken from Arizona Canal at Mesa, Ariz., 1899–1900* [1]

Period.	Calcium plus magnesium.	Sodium plus potassium.	Calcium-sodium ratio.
	P. p. m.	*P. p. m.*	
1. High and low summer water, Aug., 1899	85.3	134.9	39 : 61
2. Summer flood water, Sept. 2 to 9, 1899	125.3	197.1	39 : 61
3. High and low summer water, Sept. 10 to Oct. 9, 1899	100.3	285.4	26 : 74
4. Low winter water, Oct. 18 to Dec. 30, 1899	68.6	325.2	21 : 79
5. Low winter water, Feb. 17 to May 30, 1900	72.9	339.3	21 : 79
6. Very low summer water, June 1 to Aug. 4, 1900	97.9	418.5	23 : 77

[1] FORBES, R. H. THE RIVER-IRRIGATING WATERS OF ARIZONA. Ariz. Agr. Exp. Sta. Bul. 44, p. 174. 1902.

TABLE VII.—*Analysis of Gila River water samples taken at head of Florence Canal, 1899–1900* [1]

Period.	Calcium plus magnesium.	Sodium plus potassium.	Calcium-sodium ratio.
	P. p. m.	*P. p. m.*	
1. Low winter water, Nov. 28, 1899 to Jan. 18, 1900	78.8	329.9	24 : 76
2. Low winter water, Feb. 1 to Mar. 7, 1900	95.2	294.5	24 : 76
3. Summer flood water, Sept. 1 to 28, 1900	69.2	104.9	40 : 60
4. Summer low water, Sept. 29 to Nov. 5, 1900	118.5	286.9	29 : 71

[1] FORBES, R. H. OP. CIT., p. 192.

TABLE VIII.—*Analysis of Colorado River water samples taken at Yuma, Ariz., 1900–1901* [1]

Period.	Calcium plus magnesium.	Sodium plus potassium.	Calcium-sodium ratio.
	P. p. m.	*P. p. m.*	
1. Low winter water, Jan. 10 to Mar. 26, 1900	106.3	200.5	35 : 65
2. Rising summer water, Mar. 27 to Apr. 31, 1900	68.6	174.2	28 : 72
3. High summer water, May 1 to June 29, 1900	53.8	65.1	45 : 55
4. Low summer water, June 30 to Aug. 26, 1900	57.8	89.2	39 : 61
5. Summer flood water (local), Aug. 1 to Oct. 1, 1901	98.7	164.0	38 : 62
6. Summer flood water (Ariz.) Oct. 2 to Nov. 19, 1900	152.5	203.0	43 : 57
7. Low winter water, Nov. 20, 1900, to Jan. 24, 1901	119.7	171.7	41 : 59

[1] FORBES, R. H. OP. CIT., p. 205.

These data with respect to some of the more important irrigation streams of the western United States show that in general these waters carry a higher proportion of sodium than the Nile. If our present hypothesis is correct it may be found advisable to remedy this condition by supplying calcium, or some other high valent base such as aluminum, in soluble form to the land or to the irrigation water. There are marked differences in irrigated lands under the same water supply with respect to the tendency to become impermeable with continued irrigation. Further investigation may be expected to explain these differences and may point the way to correcting difficulties that are now acute.

SUMMARY

(1) On certain irrigated lands in the western United States the soils are not readily permeable to water and become hard and difficult to work into good tilth when dry.

(2) In extreme cases such soils are relatively unproductive because they do not absorb a sufficient quantity of water from periodical irrigations to supply the crop plants, particularly during hot, dry weather.

(3) Where the soil is not readily permeable to water it is sometimes difficult to wash out the excess of soluble salts that may be present in such quantities as to injure crop plants.

(4) This hardness or impermeability of the soil is believed to be due to the effect of sodium on the clay in the soil.

(5) A soil may become hard and its permeability become reduced by irrigating with water containing sodium carbonate.

(6) Similar results may follow when soils containing excessive quantities of sodium chlorid or sodium sulphate are leached with pure water.

(7) In the presence of certain other bases such as calcium or aluminum, in appreciable quantities and in soluble form, the injurious action of sodium on the clay does not take place.

(8) When irrigation water contains more sodium and potassium than calcium and magnesium there is danger that its continued use may cause the land to become hard and impermeable to water.

(9) Some of the important supplies of irrigation water in the United States carry more sodium and potassium than calcium and magnesium, and difficulties of hardness and impermeability of the soils are developing from the use of such water.

(10) It is believed that these difficulties may be remedied through the use of calcium or aluminum in soluble form applied either to the land or in the irrigation water.